F0RMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERES OF VENLAFAXINE HCL USING THREE FACTORS AT TWO LEVEL FULL FACTORIAL DESIGN

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CONTENTS

1.0 ABSTRACT

The objective of the present investigation was to prepare, evaluate and optimize mucoadhesive microspheres of Venlafaxine HCl. 8 deferent formulations of Venlafaxine HCl microspheres were prepared by emulsion solvent evaporation method employing three polymers (EC, Eudragit RS 100, HPMC K4M) at different ratio using a magnetic stirrer (800rpm). Acetone was used as solvent, light liquid paraffin as continues system and Span 80 was used as emulsifier (1%w/v).the prepared microspheres were evaluated for percentage yield, Particle size distribution, Angle of repose, Bulk density, Drug content, Encapsulation efficiency, Shape and Surface characterization, In vivo wash–off test, In vitro dissolution studies and drug release mechanism was interpreted by kinetic models and formulation was optimized by f_2 similarity factor. The effect of polymer on drug release and mucoadhesion was investigated. The physico- chemical properties was satisfactory for prepared formulations All the formulations exhibited anomalous (non-fickian transport) diffusion mechanism and follow first order kinetics. The formulation F_5 (-EC-750 mg Eudragit RS100 100 mg, HPMC K4M-300 mg,) was selected as optimized formulation; with 93.08% entrapment efficiency, good mcoadhesive strength and 82.58% of drug release at 24^{th} hours. Finally it is concluded that with limited number of experiments an optimized formulation with target release and good mucoadhesion can be developed with appropriate statistical experimental design.

2.0 INTRODUCTION

The concept of drug delivery has been revolutionized. The studies has been made to lend patient derive maximum benefits of drug. The drug should be delivered to specific target sites at a rate and concentration that permit optimal therapeutic efficiency while reducing side effects to minimum. Another aspect to be considered in drug delivery is patient compliance during the drug delivery.

Between 1940's and 1960's the concept of chemical micro encapsulation technology is began as an alternative means of delivering drugs. In continued quest for the more refined systems, 1980's polymer technology came to be known at forefront. Further the process of targeting and site specific delivery with absolute accuracy can be achieved by attaching bioactive molecule to liposome, bioerodable polymer, implants monoclonal antibodies and various particulate carriers. The micro particle delivery system are considerable and accepted as a reliable means to deliver drug to the target site with specifically and to maintain the desired concentration at the site of interest without unto wards effects.

For many drugs a well designed drug delivery system is as important as pharmacological activities of the drug. A well designed drug delivery system can accurately deliver the drug to the side of action at desired rate and minimize its side effects by reducing exposure of drug to other tissues[1].

2.1 DEPRESSION

What is depression? [2]

Depression is a prolonged or deep emotional sensation of sadness, being "blue", or "down." Depressive feelings such as discouragement or sadness are perfectly normal if they do not become too severe or last too long. Depression becomes a clinical problem if a person's mood becomes too depressed or if the episode lasts more than two weeks. If there is any question about the severity of a person's depression, he/she should have an immediate evaluation by a mental health professional or a physician in order to rule out suicidal intent.

2.1.1. WHAT ARE THE CHARACTERISTICS ASSOCIATED WITH DEPRESSION?

Depression includes some of the following characteristics:

- Feeling sad, blue, or down
- Feeling unworthy
- Feeling guilty
- Feeling helpless
- Loss of energy
- Feeling restless
- Feeling irritable
- Feeling lethargic

- Fatigue
- Increased sleep or decreased sleep
- Insomnia or awakening during the night
- Awakening earlier or later than normal
- Loss of interest in hobbies, activities
- Loss of interest in sex
- Decreased ability to concentrate
- Decreased ability to remember
- Increase or decrease of appetite
- Increase or decrease in weight
- Thoughts of death
- Thoughts of suicide

Also, physical symptoms such as chronic pain or a lingering illness can sometimes signal a depression. Similar to other illnesses, such as heart problems, asthma, or diabetes, depression can become severe and disabling.

People with depression usually have problems with poor sleep, low mood and appetite, loss of energy and interest or pleasure etc. It is a common illness, affecting 3% of the population per year. The main theory about why this happens is called “mono amine hypothesis”. We know that serotonin and nor adrenaline in the brain are involved with control of sleep wake, emotions, mood, arousal, drive and temperature.

Thus if a person has too little serotonin and nor adrenaline in the part of the brain that controls mood this will produce too little activity and that part of the brain becomes slower and less effective. This will lower mood.

In depression it is known that there are reduced levels of serotonin and nor adrenaline. These reduced levels lead to lowering of mood. The full reasons are not fully known but stress may well play a part in causing this.

2.1.2 ETIOLOGY OF DEPRESSION [3]

The etiology of depression, the mood disorder most frequently studied, is far from ideally understood. Many cases of depression are triggered by stressful life events, yet not everyone becomes depressed under such circumstances. The intensity and duration of these events, as well as each individual's genetic endowment, coping skills and reaction, and social support network contribute to the likelihood of depression. That is why depression and many other mental disorders are broadly described as the product of a complex interaction between biological and psychosocial factors. The relative importance of biological and psychosocial factors may vary across individuals and across different types of depression.

This section of the chapter describes the biological, genetic, and psychosocial factors—such as cognition, personality, and gender—that correlate with, or predispose to, depression. The discussion of genetic factors also incorporates the latest findings about bipolar disorder. Genes are implicated even more strongly in bipolar disorder than they are in major depression, galvanizing a worldwide search to identify chromosomal regions where genes may be located and ultimately to pinpoint the genes themselves (NIMH, 1998).

2.1.3 DIAGNOSIS AND TREATMENT [4]

Diagnosis and treatment of the sever anxiety disorders has advanced recently .Stimulated by the discovery that selective serotonin-reuptake inhibitors which are effective antidepressants also are powerful anti anxiety agents. Disorders include panic agoraphobia; social and other phobias, generalized anxiety and obsessive-compulsive disorder all appear to be responsive to treatment with serotonin reuptake inhibitors.

The process of development for DSM-IV published by the APA in 1994, was guided by specific reviews based upon new empirical evidence for diagnosis. In the classification of mood disorders, the major change in DSM-IV from DSM-III and DSM-III-R includes a listing of nine criterion symptoms of which dysphoric mood or depressed mood or loss of interest or pleasure must be present nearly every day most of the day during a 2-week period. Four additional symptoms associated with a primary depressed mood or loss of interest must be met. Previously, in DSM-III, the criteria of depressed mood or loss of interest were listed as criterion A and four of eight additional symptoms were required for the diagnosis of major depressive episode.

More substantial changes were made in the diagnostic criteria for Dysthymic Disorder. In DSM-III, the diagnosis of depressive mood required 2 years duration and three of 13 criteria in the absence of psychotic symptoms or another pre-existing mental disorder. In DSM-IV, depressed mood most of the day for at least 2 years was required in the presence of two of six criterion symptoms. The exclusion criteria again included a chronic psychotic disorder but other common psychiatric disorders did not pose specific exclusion criteria in diagnosis. In DSM-IV, clinically significant distress or impairment in social, occupational or other important areas in functioning was required. In general, DSM-IV permitted more co-occurring diagnoses to be listed on Axis I without specific

exclusion factors. An additional difference in DSM-IV represents the diagnosis of secondary mood disorders, characterized as Mood Disorder Due to a General Medical Condition or Substance- Induced Mood Disorder, in which the disturbance in mood is judged to be a direct effect of a General medical condition or due to substance intoxication, withdrawal or other medication use. The clinician then specifically notes the name of the general medical Condition on Axis I or the specific substance involved in intoxication or withdrawal

Treatment of Depression.

The three for a fuller discussion of standard, rarer and more experimental treatments, see most commonly indicated treatments for depression are psychotherapy, medication, and electroconvulsive therapy. Psychotherapy is the treatment of choice for people under 18, while electroconvulsive therapy is only used as a last resort in severe cases or in emergencies. Patients are usually assessed and managed as outpatients, and only admitted to an inpatient mental health unit if they are considered to pose a risk to themselves or others.

1 Psychotherapy

Albert Ellis invented Rational Emotive Behavior Therapy, the earliest form of Cognitive Behavioral Therapy.

There are a number of different psychotherapies for depression, which may be provided to individuals or groups. Psychotherapy can be delivered by a variety of mental health professionals, including psychotherapists, psychiatrists, psychologists, clinical social workers, counselors, and psychiatric nurses. With more complex and chronic forms of depression the most effective treatment is often considered to be a combination

of medication and psychotherapy. In people under 18, medication is offered only in conjunction with psychotherapy, not as a first line agent.

The most studied form of psychotherapy for depression is cognitive behavioral therapy (CBT), thought to work by teaching clients to learn a set of useful cognitive and behavioral skills. Earlier research suggested that cognitive-behavioral therapy was not as effective as antidepressant medication; however, more recent research suggests that it can perform as well as antidepressants in patients with moderate to severe depression.

For the treatment of adolescent depression, CBT performed no better than placebo, and significantly worse than the antidepressant fluoxetine. Combining fluoxetine with CBT appeared to bring no additional benefit or, at the most, only marginal benefit.

Two randomized, controlled trials of mindfulness-based cognitive therapy (MBCT), which includes elements of meditation, have been reviewed. MBCT was significantly more effective than "usual care" for the prevention of recurrent depression in patients who had had three or more depressive episodes. According to the review, the "usual care" did not include antidepressant treatment or any psychotherapy, and the improvement observed may have reflected the non-specific or placebo effects.

Interpersonal psychotherapy focuses on the social and interpersonal triggers that may cause depression. There is evidence that it is an effective treatment for depression. Here, the therapy takes a structured course with a set number of weekly sessions (often 12) as in the case of CBT; however the focus is on relationships with others. Therapy can be used to help a person develop or improve interpersonal skills in order to allow him or her to communicate more effectively and reduce stress.

Psychoanalysis, a school of thought founded by Sigmund Freud that emphasizes the resolution of unconscious mental conflicts, is used by its practitioners to treat clients presenting with major depression. A more widely practiced, eclectic technique, called psychodynamic psychotherapy, is loosely based on psychoanalysis and has an additional social and interpersonal focus. In a meta-analysis of three controlled trials, psychodynamic psychotherapy was found to be as effective as medication for mild to moderate depression.

2 Electroconvulsive therapy

Electroconvulsive therapy (ECT) is a procedure where seizures are electrically induced in anesthetized patients for therapeutic effect. ECT is most often used as a "last resort" (from the perspective of hospital psychiatrists) for severe major depression which has not responded to trials of antidepressant or, less often, psychotherapy or supportive interventions. It has a quicker effect than antidepressant therapy, and thus may be the treatment of choice in emergencies such as catatonic depression where the patient has ceased oral intake of fluid or nutrients, or where there is severe suicidality. Some evidence suggests it is the most effective treatment for depression in the short-term and one study without a comparison group or assessment of additional treatments given, suggested remission is related to improved self-rated quality of life in both the short-term (correlated with the degree of amnesia) and after six months. However, ECT has been found to have much lower remission rates in real-world practice and on its own does not have a sustained benefit as nearly everyone relapses. The relapse rate in the first six months may be reduced by the use of psychiatric medications or further ECT (though the latter is not recommended by some authorities, such as NICE), but remains high. Short-term memory loss, disorientation, headache and other adverse effects are common, as are

long-term memory and other neurocognitive deficits, which may persist. The American Psychiatric Association and the National Institute for Health and Clinical Excellence have concluded that the evidence they had suggested that the procedure, when administered according to their standards and without complications, does not cause brain damage in adults.

3 Other methods of treatment

Several other less widely used treatments have been officially approved either in the US or Europe. St. John's wort extract is available as a prescription antidepressant in several European countries, but is classified as an herbal supplement and sold over the counter in the US. A systematic meta-analysis of 37 trials conducted by Cochrane Collaboration indicated statistically significant weak-to-moderate effect compared to placebo. The same meta-analysis found that St John's wort efficacy for major depression is not significantly different from that of prescription antidepressants. NCCAM and other NIH-affiliated organizations hold that St. John's wort has minimal or no effects beyond placebo in the treatment of major depression, based primarily on one study with negative outcome conducted by NCCAM. S-Adenosyl methionine (SAM-e) is another drug available as a prescription antidepressant in Europe, and as an over-the-counter dietary supplement in the US. Fairly strong evidence from 16 clinical trials indicates it to be as effective as standard antidepressant medication for the treatment of major depression.

In repetitive trans-cranial magnetic stimulation (rTMS), powerful magnetic fields are applied to the brain from outside the head. Multiple controlled studies support the use of this method in treatment-resistant depression; it has been approved for this indication in Europe, Canada and Australia, but not in the US. A 2008 meta-analysis based on 32 trials found a robust effect of this method on depression, and it appeared similarly

effective for both uncomplicated depression and depression resistant to medication. However, it was inferior to ECT in a side-by-side randomized trial.

A number of other therapeutic approaches have sometimes been used although they are not officially sanctioned. Bright light therapy has been found to be an effective treatment for the winter depression produced by seasonal affective disorder. In the analysis commissioned by APA it appeared to be moderately effective for non-seasonal depression, although it did not improve the outcome when combined with standard antidepressant therapy. Another meta-analysis of light therapy for non-seasonal depression conducted by Cochrane Collaboration studied a different set of trials, in which light was used mostly as an addition to medication or sleep deprivation. A moderate statistically significant effect was found, although it disappeared if a different statistical technique was used. Both analyses noted poor quality of most studies and their small size and urged caution in the interpretation of their results. The short duration (1–2 weeks) of most trials makes it unclear whether the effect of light therapy could be sustained in the longer term.

Many people who regularly exercise have an intuitive sense that the activity is beneficial to their mood states. Some empirical support for this comes from a Duke University study, in which exercise, when used in conjunction with medication by non-suicidal patients, had beneficial effects in preventing the return of depression. Patients who completed 30 minutes of brisk exercise at least three times a week were found to have a significantly lower incidence of relapse.

Epidemiological studies indicate that the countries with high consumption of omega-3 fatty acids may have a lower rate of depression. For major depression, omega-3 fatty acid have been studied primarily as an adjunct to antidepressant therapy. A meta-

analysis of eight such trials indicated a statistically significant superiority of combinations with omega-3 fatty acids over single antidepressants; however, the authors warned that, due to multiple problems with these trials, a reliable conclusion was difficult to achieve. Tryptophan and 5-hydroxytryptophan (5-HTP) are obvious candidates for antidepressants, because they are metabolic precursors for serotonin. The Cochrane Collaboration analyzed the combined set of trials for the both of these treatments. Although the results appeared to indicate better than placebo efficacy, only two out of 108 trials were of sufficient quality to be included in the analysis.

Several other treatments deserve a brief mention. Acupuncture has been tried, but a 2004 Cochrane Review concluded that there was insufficient evidence to judge its effectiveness for managing depression. Deep brain stimulation is currently in a very early investigational stage, and data are available only from a handful of case studies. Vagus nerve stimulation is an FDA-approved therapy for treatment-resistant depression. However, the support for this method comes mainly from open-label trials and the only large double-blind trial yielded inconclusive results

2.1.4 ANTI DEPRESSANTS [5,6]

These are drugs which can elevate mood in depressive illness. Practically all antidepressants affect monoaminergic transmission in the brain in one way or the other and many of them have other associated properties The treatment of depression relies on a varied group of anti depressant therapeutic agents in part because clinical depression is a complex syndrome of widely varying severity. The first agent used successfully were tricyclic antidepressants which elicit a wide range of neuro pharmacological effects in addition to their presumed primary action .i.e. inhibiting nor epinephrine uptake into nerve endings and thus leading to sustained facilitation of

noradrenergic and perhaps serotonergic function in the brain. Inhibitors of monoamine oxidase, which increases the brain concentration of many amines, also have been used. Particularly over the past two decades a large number of anti depressants with an assortment of effects of reuptake /metabolism of biogenic amine and on pre /post-junctional aminergic/cholinergic receptors have become available so that a cogent classification is difficult. The following working classification may be adopted(Table 1).

Table 1

NAME OF DRUG	DOSE in mg	MECHANISM OF ACTION
Moclobamide	300-600	Reversible inhibitors of MAO-A
Clorgyline	300-600	Reversible inhibitors of MAO-A
Imipramine	50-200	Tri cyclic Antidepressant inhibits NA+5-HT reuptake
Amitriptyline	50-200	Tri cyclic Antidepressant inhibits NA+5-HT reuptake
Trimipramine	50-150	Tri cyclic Antidepressant inhibits NA+5-HT reuptake
Doxepin	50-150	Tri cyclic Antidepressant inhibits NA+5-HT reuptake
Dothiepin	50-150	Tri cyclic Antidepressant inhibits NA+5-HT reuptake
Clomipramine	50-150	Tri cyclic Antidepressant inhibits NA+5-HT reuptake
Desipramine	50-200	Tri cyclic Antidepressant inhibits NA reuptake
Nortriptyline	50-150	Tri cyclic Antidepressant inhibits NA reuptake
Amoxapine	100-300	Tri cyclic Antidepressant inhibits NA reuptake
Fluoxetine	20-60	Selective serotonin reuptake inhibitors
Fluoxamine	50-200	Selective serotonin reuptake inhibitors
Paroxetine	20-50	Selective serotonin reuptake inhibitors
Setraline	50-200	Selective serotonin reuptake inhibitors
Citalopram	20-40	Selective serotonin reuptake inhibitors
Venlafaxine	75-150 Serotonin/no r adrenaline reuptake inhibitors	
Trazodone	50-200	Weak inhibitors of 5-HT reuptake and α-blocker
Mianserin	30-100	Presynaptic α2-blocker
Mirtazapine	15-45	Presynaptic α2-blocker
Tianeptine	20-60	5-HT reuptake inhibitor

2.1.4.1 SOME KEY FACTS ABOUT ANTIDEPRESSANTS [5]

- The symptoms of depression are caused by an imbalance of chemicals in the brain probably reduced levels of serotonin and nor adrenaline.
- Anti depressants help correct this imbalance in the brain.
- Antidepressants are not stimulants
- They do not alter personality
- They are not additive and are not habit forming

- They do not loose their effect if you keep taking them

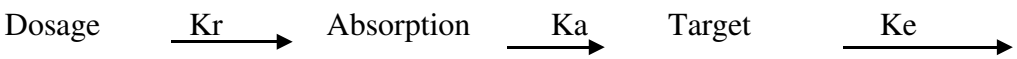
There are lots of theories about how depression occurs e.g. genetics, how the brain develops, stress etc. There are many in fact be many cause and in each person there may a combination of these. Stress may in fact cause changes in the brain which then result in reduced levels of serotonin and nor adrenaline. Transmitters other than much serotonin and nor adrenaline are probably also involved.

2.1.4.2 ANTIDEPRESSANTS ACTION

If too little serotonin produces the symptoms of depression the correcting this should helps to reduce symptoms. One way of doing this is to block the reuptake of transmitters. This is just what these antidepressants do. They block the reuptake of serotonin and nor adrenaline, so the next time an impulse comes along, there is more transmitters stronger message is passed, and activity in that part of the brain is increased. The important thing to remember is that tricyclic and related antidepressants probably mainly work by correcting the effect of having too little transmitter. They are not just stimulants

2.2 SUSTAINED RELEASE DRUG THERAPY [7,5]

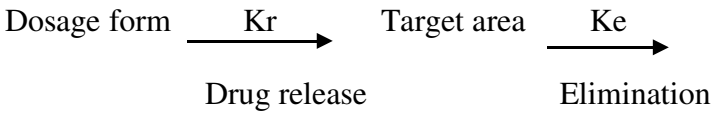
Conventional dosage forms include solutions, suspensions, capsules, tablets, emulsions, aerosols, foams, ointments and suppositories. These dosage forms can be considered to release their active ingredients into an absorption pool immediately. This is illustrated in the following simple kinetic scheme:



form drug release Pool absorption Area Elimination

The absorption pool represents a solution of the drug at the site of absorption, and the terms Kr, Ka and Ke are first – order rate constants for drug release, absorption and overall elimination, respectively. Immediate release from a conventional dosage form implies that Kr>>>Ka or that observation of drug across a biological membrane, such as the intestinal epithelium, is the rate-limiting step in delivery of the drug to its target area.

For non immediate – release dosage form, Kr<<<Ka, that is, release of drug from the dosage form is the rate - limiting step. This causes the above kinetic to reduce the following:



To overcome the potential problems associated with conventional drug therapy, modified release/ non-immediate-release delivery systems were developed and may be divided into four categories:

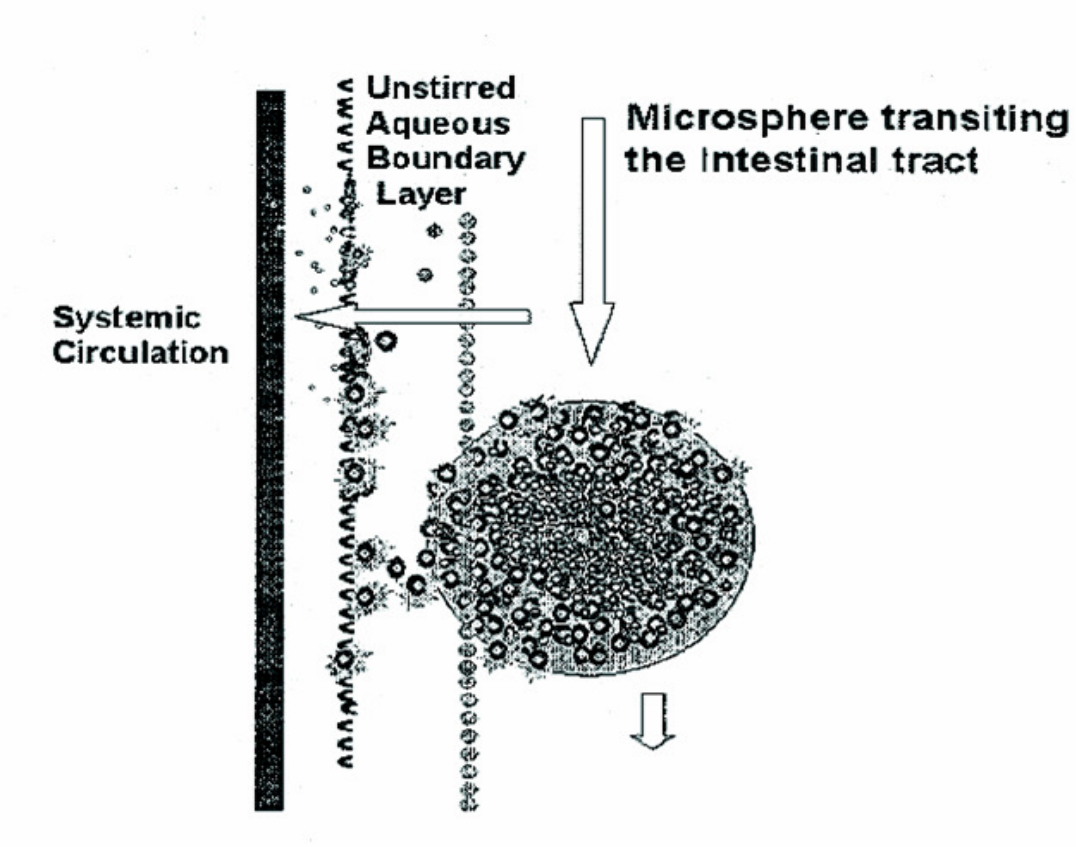
1. Delayed release
2. Sustained release
    - Controlled release
    - Prolonged release
3. Site-specific release
4. Receptor release

2.3. MUCOADHESIVE MICROSPHERES

Mucoadhesive microspheres include micro particles and microcapsules (having a core of the drug) of 1-1000 μm in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it, respectively[8]. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of mucoadhesive properties to microspheres has additional advantages, *e.g.* efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of drug to the absorption site achieved by anchoring plant lectins[9], bacterial adhesions[10] and antibodies[11], *etc.* on the surface of the microspheres. Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye, nasal cavity and urinary and gastrointestinal tract, thus offering the possibilities of localized as well as systemic controlled release of drugs. Application of mucoadhesive microspheres to the mucosal tissues of ocular cavity, gastric and colonic epithelium is used for administration of drugs for localized action. Prolonged release of drugs and a reduction in frequency of drug administration to the ocular cavity can highly improve the patient compliance[12]. The latter advantage can also be obtained for drugs administered intra-nasally due to the reduction in mucociliary clearance of drugs adhering to nasal mucosa[13]. Microspheres prepared with mucoadhesive and bioerodible polymers undergo selective uptake by the M cells of Payer patches in gastrointestinal (GI) mucosa[14]. This uptake mechanism has been used for the delivery of protein and peptide drugs, antigens for vaccination and plasmid DNA for gene therapy. Moreover, by keeping the drugs in close proximity to their absorption window in the GI mucosa. The mucoadhesive microspheres improve the absorption and oral bioavailability of drugs like furosemide[15] and riboflavin[16]. The

concept of a non-invasive single shot vaccine, by means of mucosal immunization, offers controlled release of antigens and thus forms another exquisite application of mucoadhesive microspheres[17]

2.3.1 POLYMERS USED FOR MUCOADHESIVE MICROSPHERES



The type of polymers used to prepare them influences the properties of the mucoadhesive microspheres, based on their surface characteristics, force of mucoadhesion, release pattern of the drug, and clearance. Suitable polymers that can be used to form mucoadhesive microspheres include soluble and insoluble, non-biodegradable and biodegradable polymers. These can be hydrogels or thermoplastics, homopolymeres, copolymers or blends, natural or synthetic polymers.

2.3.2 CLASSIFICATION OF POLYMERS

(a) ***Hydrophilic Polymers***

These are the water-soluble polymers that swell indefinitely in contact with water and eventually undergo complete dissolution, Eg: Methylcellulose, hydroxyethyl cellulose, hydroxy propyl methyl cellulose, sodium carboxy methyl cellulose, carbomers, chitosan and plant gums *etc.*

(b) Hydrogels

These are water swellable materials, usually a cross-link polymer with limited swelling capacity, eg: Poly acrylic acid co acrylamide copolymers, sodium alginate, guar gum and modified guar gum *etc.*

(c) ***Thermoplastic Polymers***

These polymers include the non-erodible neutral polystyrene and semi crystalline bioerodible polymers, which generate the carboxylic acid groups as they degrade, *Eg*: polyanhydrides and polylactic acid. Various synthetic polymers used in mucoadhesive formulations include polyvinyl alcohol, polyamides, polycarbonates, polyalkylene glycols, polyvinyl ethers, esters, halides, polymethacrylic acid, polymethylmethacrylic acid, methylcellulose, hydroxypropyl cellulose, and hydroxypropyl methylcellulose and sodium carboxymethylcellulose. Various biocompatible polymers used in mucoadhesive formulations include cellulose-based polymers, ethylene glycol polymers and its copolymers, oxyethylene polymers, polyvinyl alcohol, polyvinyl acetate and esters of haluronicacid. Various biodegradable polymers used in mucoadhesive formulations are poly lactides, poly glycolides, poly lactide-co-glycolides, polycaprolactones, and

polyalkyl cyanoacrylate. Polyorthoesters, polyphosphoesters, polyanhydrides and polyphosphazenes are the recent additions to the polymers.

2.3.3 METHODS OF PREPARATON OF MUCOADHESIVE MICROSPHERES

Mucoadhesive microspheres can be prepared using any of the following techniques.

(a) ***Solvent Evaporation***

It is the most extensively used method of microencapsulation, first described by *Ogawa et al.*[18] A buffered or plain aqueous solution of the drug (may contain a viscosity building or stabilizing agent) is added to an organic phase consisting of the polymer solution in solvents like dichloromethane (or ethyl acetate or chloroform) with vigorous stirring to form the primary water in oil emulsion. This emulsion is then added to a large volume of water containing an emulsifier like PVA or PVP to form the multiple emulsions (w/o/w). The double emulsion, so formed, is then subjected to stirring until most of the organic solvent evaporates, leaving solid microspheres. The Microspheres can then be washed, centrifuged and lyophilize to obtain the free flowing and dried microspheres.

(b) ***Hot Melt Microencapsulation***

This method was first used by *Mathiowitz and Langer*[19] to prepare microspheres of polyanhydride copolymer of poly [bis (*p*-carboxy phenoxy) propane anhydride] with sebacic acid. In this method, the polymer is first melted and then mixed with solid particles of the drug that have been sieved to less than 50 µm. The mixture is suspended

in a non-miscible solvent (like silicone oil), continuously stirred, and heated to 5°C above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microspheres are washed by decantation with petroleum ether. The primary objective for developing this method is to develop a microencapsulation process suitable for the water labile polymers, *e.g.* polyanhydrides. Microspheres with diameter of 1-1000 µm can be obtained and altering the stirring rate can easily control the size distribution. The only disadvantage of this method is moderate temperature to which the drug is exposed.

(c*) Solvent Removal*

It is a non-aqueous method of microencapsulation, particularly suitable for water labile polymers such as the ploy anhydrides. In this method, drug is dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent like methylene chloride. This mixture is then suspended in silicone oil containing span 80 and methylene chloride[20]. After pouring the polymer solution into silicone oil, petroleum ether is added and stirred until solvent is extracted into the oil solution. The resulting Microspheres can then be dried in vacuum.

(d) *Hydro gel Micro spheres*

Microspheres made of gel-type polymers, such as alginate, are produced by dissolving the polymer in an aqueous solution, suspending the active ingredient in the mixture and extruding through a precision device, producing micro droplets which fall into a hardening bath that is slowly stirred. The hardening bath usually contains calcium chloride solution, whereby the divalent calcium ions crosslink the polymer forming gelled microspheres. The method involves an "all-aqueous" system and avoids residual

solvents in microspheres. *Lim and Moss*[21] developed this method for encapsulation of live cells, as it does not involve harsh conditions, which could kill the cells. Coating them with polycationic polymers, like polylysine after fabrication, can further modify the surface of these microspheres. The particle size of microspheres can be controlled by using various size extruders or by varying the polymer solution flow rates.

(e) ***Spray Drying***

In this process, the drug may be dissolved or dispersed in the polymer solution and spray dried. The quality of spray-dried microspheres can be improved by the addition of plasticizers, *e.g.* citric acid, which promote polymer coalescence on the drug particles and hence promote the formation of spherical and smooth surfaced microspheres. The size of microspheres can be controlled by the rate of spraying, the feed rate of polymer drug solution, nozzle size, and the drying temperature. This method of micro encapsulation is particularly less dependent on the solubility characteristics of the drug and polymer and is simple, reproducible, and easy to scale up[22]

(f) ***Phase Inversion Micro encapsulation***

The process involves addition of drug to a dilute solution of the polymer (usually 1-5%, w/v in methylene chloride). The mixture is poured into an unstirred bath of strong non-solvent (petroleum ether) in a solvent to non-solvent ratio of 1: 100, resulting in the spontaneous production of microspheres in the size range of 0.5-5.0 mm can then be filtered, washed with petroleum ether and dried with air[23]. This simple and fast process of micro encapsulation involves relatively little loss of polymer and drug.

2.3.4 EVALUATION OF MUCOADHESIVE MICROSPHERES

The best approach to evaluate mucoadhesive microspheres is to evaluate the effectiveness of mucoadhesive polymer to prolong the residence time of drug at the site absorption, thereby increasing absorption and bioavailability of the drug. The methods used to evaluate mucoadhesive microspheres include the following.

2.3.4.1 *Measurement of Adhesive Strength*

The quantification of the mucoadhesive forces between polymeric microspheres and the mucosal tissue is a useful indicator for evaluating the mucoadhesive strength of microspheres. *In vitro* techniques have been used to test the polymeric microspheres against a variety of synthetic and natural mucus, frozen and freshly excised tissue *etc.* The different *in vitro* methods include the following.

(a) Tensile Stress Measurement (Wilhelmy Plate Technique)

The wilhelmy plate technique is traditionally used for the measurement of dynamic contact angles and involves the use of a microdensitometer or microbalance. The CAHN dynamic contact angle analyzer (model DCA 322, CAHN instruments, Cerritos) has been modified to perform adhesive micro force measurements. The DCA 322 system consists of an IBM compatible computer and microbalance assembly[24]. The microbalance unit consists of stationary sample and tare loops and a motor powered

translation stage. The instrument measures the mucoadhesive force between mucosal tissue and a single microsphere mounted on a small diameter metal wire suspended from the sample loop in microtesiometer[25]. The tissue, usually rat jejunum, is mounted within the tissue chamber containing Dulbecco's phosphate buffered saline containing 100 mg/dl glucose and maintained at the physiologic temperature. The chamber rests on a mobile platform, which is raised until the tissue comes in contact with the suspended microsphere. The contact is held for 7 minutes, at which time the mobile stage is lowered and the resulting force of adhesion between the polymer and mucosal tissue recorded as a plot of the load on microsphere versus mobile stage distance or deformation. The plot of output of the instrument is unique in that it displays both the compressive and the tensile portions of the experiment. By using the CAHN soft ware system, three essential mucoadhesive parameters can be analyzed. These include the fracture strength, deformation to failure and work of adhesion.

Fracture strength: it is the maximum force per unit surface area required to break the adhesive bond.

Deformation to failure: it is the distance required to move the stage before complete separation occurs. This parameter is dependent on the material stiffness and the intensity of strength of adhesion.

Work of adhesion: It is a function of both the fracture strength and the deformation to failure. It tends to be the strongest indicator of the bioadhesive potential. This technique allows the measurement of mucoadhesive properties of a candidate material in the exact geometry of the proposed microsphere delivery device and the use of a physiological

tissue chamber mimics the *in vivo* conditions. From a single tensile experiment, 11 mucoadhesive parameters can be analyzed out of which 3 are direct predictors of the bioadhesive potential. The CAHN instrument, although a powerful tool has inherent limitations in its measurement technique, makes it better suited for large microspheres (with a diameter of more than 300µm) adhered to tissue *in vitro*. Therefore, many new techniques have been developed to provide quantitative information of mucoadhesive interactions of the smaller microspheres.

(b) ***Novel Electromagnetic Force Transducer (EMFT)***

The EMFT is a remote sensing instrument that uses a calibrated electromagnetic to detach a magnetic loaded polymer microsphere from a tissue sample[26]. It has the unique ability to record remotely and simultaneously the tensile force information as well as high magnification video images of mucoadhesive interactions at near physiological conditions. The EMFT measures tissue adhesive forces by monitoring the magnetic force required to exactly oppose the mucoadhesive force. To test a microsphere, it must first be attached to the sample of tissue; magnetic force is then generated by an electromagnet mounted on the microscope vertically above the tissue chamber. After the computer has calculated the position of microsphere, the tissue chamber is slowly moved down, away from the magnet tip. As the tissue slowly descends away from the magnet, the video analysis continuously calculates the position of microsphere until the latter is completely pulled free of the tissue. The computer can display the results either as raw data or convert it to a force *versus* displacement graph. The primary advantage of the EMFT is that no physical attachment is required between the force transducer and the microsphere. This makes it possible to perform accurate mucoadhesive measurements on the small microspheres, which have been implanted

in vivo and then excised (along with the host tissue) for measurement. This technique can also be used to evaluate the mucoadhesion of polymers to specific cell types and hence can be used to develop mucoadhesive drug delivery system (MDDS) to target- specific tissues[27].

(c) ***Shear Stress Measurement***

The shear stress measures the force that causes a mucoadhesive to slide with respect to the mucus layer in a direction parallel to their plane of contact[28]. Adhesion tests based on the shear stress measurement involve two glass slides coated with polymer and a film of mucus. Mucus forms a thin film between the two polymer coated slides, and the test measures the force required to separate the two surfaces. *Mikos and Peppas*[29] designed the *in vitro* method of flow chamber. The flow chamber made of Plexiglas is surrounded by a water jacket to maintain a constant temperature. A polymeric microsphere placed on the surface of a layer of natural mucus is placed in a chamber. A simulated physiologic flow of fluid is introduced in the chamber and movement of microsphere is monitored using video equipment attached to a goniometry, which also monitors the static and dynamic behavior of the micro particles.

(d) ***Adhesion Number***

Adhesion number for mucoadhesive microspheres is determined as the ratio of the number of particles attached to the substrate to the total number of applied particles, expressed as a percentage. The adhesion strength increases with an increase in the adhesion number.

(e) ***Falling Liquid Film Method***

It is a simple, quantitative in situ method, wherein an excised intestinal segment cut lengthwise, is spread on a plastic flute and positioned at an incline. The suspension of microsphere is allowed to flow down the intestinal strip. Particle concentrations entering the segment from the dilute suspension reservoir and leaving the intestinal segment can be determined with the help of coulter counter to quantify the steady state fraction of particles adhered to the intestinal mucosa. The percent of particles retained on the tissue is calculated as an index of mucoadhesion[30].

(f) *Everted Sac Technique*

The everted intestinal sac technique is a passive test for mucoadhesion and involves polymeric microspheres and a section of the everted intestinal tissue. It is performed using a segment of intestinal tissue excised from the rat, everted, legated at the ends and filled with saline. It is then introduced into a tube containing a known amount of the microspheres and saline, and agitated while incubating for 30 min. Sac is then removed, microspheres are washed and lyophilized, and the percentage of binding to the sac is calculated from difference in the weight of the residual spheres from the original weight of the microspheres[31]. The advantage of the technique is that no external force applied to the microspheres being tested; microspheres are freely suspended in buffer solution and made to come in contact with the everted intestinal tissue randomly. The CAHN technique and the everted intestinal sac technique, both predict the strength of mucoadhesion in a very similar manner. Santos *et al* established a correlation between the two *in vitro* mucoadhesion assay methods, which thereby allows one to confidentially utilize a single mucoadhesion assay to scan a variety of mucoadhesive polymers. [75]

INVIVO TECHNIQUES

(g) *Measurement of the Residence*

Time measurements of the residence time of mucoadhesive at the application site provide quantitative information on their mucoadhesive properties. The GI transit times of many mucoadhesive preparations have been examined using radioisotopes and fluorescent labeling techniques.

(h) ***GI Transit Using Radio-Opaque Microspheres***

It is a simple procedure involving the use of radio-opaque markers, *e.g.* barium sulfate, encapsulated in mucoadhesive polymers to determine the effects of mucoadhesive polymers on GI transit time. Feces collection (using automated feces Collection machine) and X-ray inspection provide a non-invasive method of monitoring total GI residence time without affecting normal GI motility. Mucoadhesives labeled with Cr-51, Tc-99m, In-113m, or I-123 have been used to study the transit of the microspheres in the GI tract.

(i) ***Gamma Scintigraphy Technique***

Distribution and retention time of the mucoadhesive intravaginal Microspheres can be studied using the gamma scintigraphy technique. A study has reported the intensity and distribution of radioactivity in the genital tract after administration of technetium labeled hyaluronic acid esters microspheres. Dimensions of the vaginal cavity of the sheep can be outlined and imaged using labeled gellan gum and the data collected is subsequently used to compare the distribution of radio labeled HYAFF formulations. The retention of mucoadhesive-radio labeled micro spheres based on HYAFF polymer was found[32] to be more for the dry powder formulation than for the

pessary formulation after 12 hours of administration to vaginal epithelium. The combination of sheep model and gamma scintigraphy method has been proved to be an extremely useful tool for evaluating the distribution, spreading and clearance of vaginally administered mucoadhesive drug delivery system, including microbicides.

2.3.4.2 *Surface Characterization of the Mucoadhesive Microspheres*

Surface morphology of microspheres and the morphological changes produced through polymer degradation can be investigated and documented using scanning electron microscopy (SEM), electron microscopy and scanning tunneling microscopy (STM). To assess the effect of surface morphology on the mucoadhesive properties, the microsphere samples are lyophilized and analyzed under SEM at 150 µm and 1000 µm. The smooth texture of the microsphere surface leads to weak mucoadhesive properties, while the coarser surface texture improves the adhesion through stronger mechanical interactions[41]. The morphological surfaces changes occurring due to the hydrolytic degradation of the polymers, e.g.: polyanhydrides can be studied after incubating the microspheres in the PBS buffer for different intervals of time.

Table 2

SUMMARY OF RESEARCH WORK ON MUCOADHESIVE MICROSPHERES AND MICROCAPSULES

DRUG	POLYMER	ROUTE	PURPOSE/RESULT
Furosemide	AD-MMS (PGEFs)	GI	Increased bioavailability Higher *AUC*
Riboflavin	AD-MMS (PGEFs)	GI	—
Amoxicillin	AD-MMS (PGEFs)	GI	Greater anti *H. pylori* activity

Delapril Hcl (prodrug) [33]	PGEFs	GI	MRT of drug is increased plasma concentrations of the active metabolite are sustained
Amoxicillin[34]	Polycarbopol/Carbopol 934 /Ion exchange resin	GI	Greater anti *H. pylori* activity
Cephradine[35]	Chitosan/ Ethyl cellulose	GI	Prolonged the intestinal absorption
Indomethacin[36]	Sodium Alginate CMC/MC/Carbopol/HPMC	Oral	Slow release rates
Glipizide[37]	Sodium Alginate CMC/MC/Carbopol/HPMC	Oral	Slow release rates

AD-MMS : Adhesive Micro matrix System

AUC : Area under Curve

MRT : Mean Residence Time

2.4 STATISTICAL OPTIMIZATION TECHNIQUE

2^3 Factorial Design [38]

The main objective of a factorial experiment is to be able to determine (or) at least the estimate the factor effects, which indicates how each factor affects the process output. Factor effects need to be understood so that the factors can be adjusted to optimize the process output. The effect of each factor on the output can be due to it alone (main effect of the factor), (or) a result of the interaction between the factor and one (or) more of the other factor (interactive effects). When assessing factor effects (whether main (or) interactive effects), one needs to consider not only the magnitudes of the

effects, but their directions as well. The directions of an effect determine the direction in which factors need to be adjusted in a process in order to optimize the process output.

In factorial designs, the main effects are referred to using single uppercase letters, (e.g.) the main effects of factor A, B&C is referred to simply as A, B&C respectively. An interactive effect, on the other hand, is referred to by a group of letters denoting which factors are interacting to produce the effect, (e.g.,) the interactive effect produced by factors A, B&C is referred to as ABC.

The magnitude and polarity (or direction) of the numerical values of main and interactive effects indicates how this affects influences the process output. A higher absolute value for an effect means that the factor responsible for it affects the out put significantly. A negative value means that increasing level(s) of the factor (s) responsible for that effect will decrease the output of the process. (Table 2 & 3)

Design experiments

Table. 3

Levels of Factors

Factor	Low level	High level
A	-	+
B	-	+
C	-	+

Table 4.

Two- level-3-factor- full-factorial Experiment design pattern

Run	Combination	*Factors*		
		A	B	C
1	(1)	-	-	-
2	A	+	-	-
3	B	-	+	-
4	AB	+	+	-
5	C	-	-	+
6	AC	+	-	+
7	BC	-	+	+
8	ABC	+	+	+

3.0 LITERATURE REVIEW

Mucoadhesive microspheres include micro particles and microcapsules (having a core of the drug) of 1-1000 µm in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it, respectively[8]. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of mucoadhesive properties to microspheres has additional advantages, *e.g.* efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of drug to the absorption site achieved by anchoring plant lectins[9], bacterial adhesions[10] and antibodies[11], *etc.* on the surface of the microspheres. Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye, nasal cavity and urinary and gastrointestinal tract, thus offering the possibilities of localized as well as

systemic controlled release of drugs. Application of mucoadhesive microspheres to the mucosal tissues of ocular cavity, gastric and colonic epithelium is used for administration of drugs for localized action. Prolonged release of drugs and a reduction in frequency of drug administration to the ocular cavity can highly improve the patient compliance[12]

Amoxicillin mucoadhesive Microspheres[39] were prepared using ethyl cellulose (EC) as matrix and carbopol 934P as mucoadhesive polymer for the potential use of treating gastric and duodenal ulcers, which were associated with *Helicobacter pylori. In vitro* release test was done in pH 1.0 (HCl) and in pH 7.8 phosphate buffer. In conclusion, the prolonged gastrointestinal residence time and enhanced amoxicillin stability resulting from the mucoadhesive microspheres of amoxicillin might make contribution to *Helicobacter pylori* clearance.

Mucoadhesive microspheres prepared[40] containing either amoxicillin or clarithromycin by interpolymer complexation of poly acrylic acid (PAA) with poly vinyl pyrrolidone (PVP) and solvent diffusion method. The loading efficiency of clarithromycin in the complex microspheres was higher than that of amoxicillin due to the stronger interaction of clarithromycin with the PAA. The microspheres had a spherical shape with a smooth surface and the inside of the microspheres was completely filled. The release mechanism of amoxicillin was mainly by a diffusion process and that of clarithromycin was via a dissolution process.

Venlafaxine extended-release capsules[41] were studied in panic disorder. Venlafaxine ER was not associated with a greater proportion of patients free from full-symptom panic attacks at the final on-therapy evaluation, but was associated with lower mean panic attack frequency and a higher proportion free from limited-symptom panic attacks, higher response and remission rates, and improvements in anticipatory anxiety,

fear and avoidance. Adverse events were comparable with those of the drug in depression and anxiety. Sustained release Venlafzxine HCl wax matrix tablets[84] was prepared and optimized; the effect of Bees wax and Carnauba wax on the drug release profile was investigated. Both waxes retarded release but Bees wax showed significant influence.

Indion 244-Venlafaxine HCl complexes [83] were prepared the optimum pH,drug loading efficiency were determined which changed crystalline character of the drug to amorphous form and sustained formulation. Method of manufacturing sustained release micro beads containing venlafaxine HCl[42] were studied. A sustained release venlafaxine composition that includes of a non-agglomerated, uniformly shaped and sized micro beads of inert core particles having a first coating layer in a concentration of at least about 5% to about 70% by weight of the composition, the binder is present in an amount of at least 35% by weight of the active agent, or in a further layer located upon or below the first coating layer, or as an alternating layer between plural first layers. The prepared sustained release microbeads containing Venlafaxine HCl used for once a management of major anxiety disorders.

Nifidepine microsphere [43] were prepared using Eudragit RL as a matrix material, by emulsion solvent evaporation method using methylene chloride as solvent; The solution was emulsified in 0.8% polyvinyl alcohol at room temperature. Thus prepared microspheres were evaluated for their drug content, particle size, distribution, bulk density, in vitro dissolution, in vivo bioavailability using cross over design and the stability study on microsphere performed at 4^{oC}, 25^{oC} and 50^{Oc}. Chlorpromazine hydrochloride (CPH) microspheres[44] were prepared using ethyl cellulose (EC) as retardant, by using solvent evaporation and non-solvent addition. In both the methods,

three different drug to polymer concentration (1:1, 1:1.5, and 1:2) were utilized for the preparation. Solvent evaporation method yielded microsphere of poly-dispersed sizes and un-uniform rough surface. Microspheres obtained from the non-solvent addition method were uniform, smooth and these were subjected for evolution. Tableting microcapsule consisting of ethyl cellulose shells or walls containing sodium Phenobarbital in the cores[45] investigated. Dissolution from the capsules was found to depend on the core-wall ratio and the size of the dried microcapsules aggregates in the tablet. With a higher core-to-wall ratio the microcapsules have thinner walls so that the dissolution medium can enter the capsules more readily and the core solution can easily pass through the walls. The larger the microcapsules the greater the breakdown of the aggregates, providing additional surface for dissolution. Thus, both core-to-wall ratio and size of microcapsule aggregates increase the dissolution rate of the tableted microcapsules.

Mucoadhesive microspheres[46], were developed that can be utilized for the controlled release of triclosan in oral-care formulations, specifically dental paste. Using a double–emulsion solvent evaporation technique, triclosan was incorporated into microspheres. Carbopol 934P, polycarbophil or chitosan and the profiles for its releases were established under simulated 'in use' conditions. The work has demonstrated that these polymeric microspheres, particularly those of chitosan, are promising candidates for the sustained release of triclosan in the oral cavity. Metoclopramide microspheres [47] prepared containing a mucoadhesive polymer chitosan and investigated with a view to develop mucoadhesive microspheres. The chitosan microspheres were prepared by simple emulsification phase separation technique using glutaraldehyde as a cross linking agent. Results of preliminary trials indicate that volume of cross-linking agent, time for cross-linking and speed of rotation affected characteristics of microspheres.

Metoclopramide release from these mucoadhesive microspheres was slow, extended and depended on the polymer to drug ratio. Drug release was diffusion controlled and followed non-Fickian diffusion. Indomethacin microcapsules with a coat[48], were formulated consisting of alginate and a mucoadhesive polymer such as sodium carboxymethylcellulose, methylcellulose, carbopol and hydroxypropylmethylcellulose were prepared by an emulsification–ionic gelation process. The resulting microcapsules were discrete, large, and spherical and free flowing. Microencapsulation efficiency was 41-70% and relatively high with alginate-sodium carboxymethylcellulose. Release from some microcapsules fulfilled the official (USP 23) drug release test-2 requirement of indomethaoin extended release capsules.

Metoprolol tartarate biodegradable microspheres[50] were prepared using chitosan by the phase separation emulsification technique. Microspheres of 1:0.5, 1:1 and 1:2 drugs to carrier ratios were prepared and thermally cross-linked. Drug to carrier ratio 1:1 showed maximum percentage yield and highest drug entrapment. The size range of the microspheres varies from 3.5 to 31.5 μm. UV and DSC studies were carried out to confirm the presence and stability of the drug in the microspheres. Short-term stability studies were carried out to confirm the presence and stability of the drug in the microspheres. Mucoadhesive properties and gastrointestinal transit of microspheres made of oppositely charged dextran derivatives and cellulose acetate butyrate (CAB) [50]., evaluated. The microspheres were prepared by emulsion solvent evaporation method. Microspheres with a diameter of 425-710 μm were examined for *in vitro* mucoadhesion by the everted sac method. The results indicated that the percentage of adherence to the rat small intestine was affected by the amount of dextran derivatives in the microspheres.

Diclofenac sodium by using sodium microspheres[51], were prepared alginate as a polymer and calcium chloride as a cross linking agent. In this investigation, 3 full factorial design was used to investigate the joint influence of three variables: the stirring speed (x_1), concentration of $CaCl_2$ (X_2), and percent of heavy liquid paraffin in a blend of heavy and light liquid paraffin in the dispersion medium (X_3) on the time required for 80% drug dissolution (t_{80}). The result of multiple linear regression analysis and F-statistics revealed that, for obtaining controlled drug release, the microsphere should be prepared using relatively lower stirring speed, higher interactions were found to be statistically significant in nature. Nitrofurantoin microspheres[52] were prepered by using three polymeric materials viz. Sodium alginate, ethyl cellulose and eudragit RS100 and reported that the microcapsules prepared with sodium alginate and ethyl cellulose demonstrated slower release than those prepared with eudragit RS100.

Ethyl cellulose microcapsules[53] was studied for its permeability which was prepared by three coacervation methods of aspirin metronidazole, paracetamol, tolbutamide; and they reported that with all the medicaments, high permeability, constants were observed in case of microcapsules prepared by thermal included coacervation and less permeability when prepared by coacervation by the addition of non-solvents. Theophylline microspheres,[54] was prepared by ionotropic gelation method and investigated the influence of variables such as stirring speed, concentration of calcium chloride, and composition of dispersion medium on the characteristics of the microsphere. The microspheres were evaluated during *in vivo* studies in dogs and the pharmacokinetics parameters were estimated by performing non-linear regression analysis. The Wagner-Nelson method was adopted to compute the percentage drug absorbed. A good correlation was observed between the *in vitro* and *in vivo* results.

Mucoadhesive properties of chitosan microspheres[55] were prepared by different methods were evaluated by studying the interaction between mucin and microspheres in aqueous solution. The interaction was determined by the measurement of mucin adsorbed on the microspheres. A strong interaction between chitosan microspheres and mucin was detected. The intensity of the interaction was dependent upon the method of preparation of chitosan microspheres and the amount of mucin added. The extent of mucus adsorption was proportional to the absolute values of the positive zeta potential of chitosan microspheres. The zeta potential in turn was found to be dependent upon the method of preparation of microspheres. The adsorption of type III mucin (1% sialic acid content) was interpreted using Freundlich or Langmuir adsorption isotherms. The values of r^2 were greater for Langmuir isotherm as compared with Freundlich isotherm. The adsorption of a suspension of chitosan microspheres in the rat small intestine indicated that chitosan microspheres prepared by tripolyphosphate cross-linking and emulsification ionotropic gelation can be used as an excellent mucoadhesive delivery system. The microspheres prepared by glutaraldehyde and thermal cross-linking showed good stability in HCl as compared with microspheres prepared by tripolyphosphate and emulsification ionotropic gelation.

The chitosan microspheres[56], were prepared by a membrane emulsification method with variations of the N_2 gas pressure and the chitosan concentration. The pressure of N_2 gas was varied within the range from0.2 X 10^5 to 0.8 X 10^5 Pa at chitosan concentration 1.5 % wt/wt. In addition, the concentration of chitosan was varied between 0.5 ~ 2.0 % wt/wt at 0.4 X 105 Pa of N_2 gas pressure. Using this method, it is possible to prepare stable emulsions with a very narrow droplet size distribution in comparison with

conventional methods. The average size of the microspheres was dependent on the N_2 gas pressure and the concentration that is it was increased with the pressure and the concentration. The modeling of the size for the microspheres according to the concentration was carried out using Macleod's relation and Parkins & Brown equation. The former shows the relationship between density and surface tension and the latter demonstrates the correlation between the volume of the microspheres and the interfacial tension. The modeling results were in good agreement with the experimental data to predict the microspheres size with the variation of concentration. Mucoadhesive microspheres were developed that can be utilized for the controlled release of triclosan[57] in oral-care formulations, specifically dental pastes. Using a double-emulsion solvent evaporation technique, triclosan was incorporated into microspheres that were prepared from Gantreze MS-955, Carbopol 974P, polycarbophil or chitosan and the profiles for its release were established under simulated 'in use' conditions. Triclosan was rapidly released into a sodium lauryl sulphate-containing buffer from all but the chitosan microspheres. The release of triclosan from microspheres suspended in a non-aqueous paste, was found to be sustained over considerable time-periods, which were influenced strongly by the nature of the polymeric carrier. For microspheres that were fabricated from Gantrez, Carbopol or polycarbophil, the release appeared to obey zero-order kinetics whereas in the case of chitosan-derived vehicles, the release profile fitted the Baker and Lonsdale model. The work has demonstrated that these polymeric microspheres, particularly those of chitosan, are promising candidates for the sustained release of triclosan in the oral cavity.

The influence of three solvents for the polymer (chloroform, dichloromethane and ethyl acetate) [58] employed in the preparation on the drug release microcapsules were

prepared by an emulsion solvent evaporation method was studied. All the three solvents gave discrete, large sized, free flowing spherical microcapsules. The microcapsules were valuated for size analysis, drug content, microencapsulation efficient, wall thickness, drug release characteristics, influence of solvent employed on diclofenac sodium release from microcapsules, surface characteristics. Diclofenac release from the microcapsules and the solvent employed in their preparation. Among the solvents employed chloroform was found to be more suitable for slow release of diclofenac from ethyl cellulose microcapsules.

Mucoadhesive microspheres [59] were formulated containing the mucoadhesive polymer chitosan hydrochloride, with matrix polymer Eudragit RS-100, pipemidic acid as a model drug and agglomeration preventing agent magnesium stearate by the solvent evaporation method. The amount of magnesium stearate was varied and the following methods were used for microsphere evaluation: sieve analysis, drug content and dissolution determination, scanning electron microscopy, X-ray diffractometry, DSC and FTIR spectroscopy. The results showed that average particle size decreased with increasing amount of magnesium stearate used for microsphere preparation. This is probably a consequence of stabilization of the emulsion droplets with magnesium stearate. Higher pipemidic acid content in the microspheres was observed in larger particle size fractions and when higher amounts of magnesium stearate were used. It was also found that these two parameters significantly influenced the dissolution rate. The important reason for the differences in drug content in microspheres of different particle sizes is the diffusion of pipemidic acid from the acetone droplets in liquid paraffin during the preparation procedure. The physical state of pipemidic acid changed from crystalline to mostly amorphous with its incorporation in microspheres, as shown by X-ray

diffractometry and differential scanning calorimetry. No differences were observed in the physical state of pipemidic acid and in microsphere shape and surface between different size fractions of microspheres, prepared with different amounts of magnesium stearate. Additionally, no correlation between the physical state of the drug in different microspheres and their biopharmaceutical properties was found.

The Spherical matrices and microcapsules of Nimesulide[60] were formulated by using ethyl cellulose (E.C) as coating material, by reported methods spherical agglomeration technique indomethacin and emulsion solvent evaporation technique for theophylline and studied the in-vitro drug release pattern from both the matrices and microcapsules of different sizes (20/40 and 40/60) and different coating thickness (10%, 20% and 25%) for about eight hours. It was found that the drug release from matrices was slow compared to that from microcapsules.

Modified release microspheres of Ibuprofen[61] were prepared by emulsion solvent diffusion method. The technique was optimized for the following processing variables the absence / presence of baffles in the reaction vessel, agitation rate and drying time. Thereafter, the influence of various formulation factors on the microencopsulotion efficiency. In vitro drug release and micromeritic properties were examined. The variables included the methacrylic polymer, Eudragit RS100, ibuprofen content and the volume of ethanol used during microencapsulation. The results obtained were interpreted on a triangular phase diagram to map the region of microencapsulation, as well as those formulations that yielded suitable modified release ibuprofen microspheres.

The forgiving literature review indicates that Venlafaxine HCl has been investigated as extended release capsules[41], sustained release was matrix tablets[84], complex with Indion 244[83], and sustained release microbeades[42]. However, to the best of our knowledge; the mucoadhesive-microspheres of Venlafaxine HCl for targeted drug delivery has not been studied, and hence the present investigation was directed towards developing the mucoadhesive microspheres of Venlafaxine HCl with greater bioavailability and enhanced loading efficiency.

4.0 AIM AND OBJECTIVE OF THE WORK

Venlafaxine HCl is a new generation anti depressant serotonine/noradrenaline reuptake inhibitor drug showing effective anti-depressant properties. It has a short bioavailability 12.6% and biological half-life of 5 hours. So, frequent administration is necessary to maintain its therapeutic concentration. This necessitates multiple daily dosing for maintenance of its plasma concentration of the drug within the therapeutic index; hence there is an impetus for developing sustained release dosage form that maintains improved bioavailability and therapeutic plasma drug concentration for long period compared to conventional dosage forms.

The objective of the present study is to design a prolonged release dosage form to be used for targeted and controlled release drug delivery, preparation of mucoadhesive microspheres containing venlafaxine HCl, helps in releasing small quantities of drug, advantage for treating of depressive disorders.

Slowly dissolving polymers for sustaining the release may be suitable for long-term therapy in controlled alleviation of clinical manifestation. Ethyl cellulose and Eudragit RS100 provide a potentially useful means of delivering drugs because they are stable, both physically and chemically amenable to preparation in large batches. However the present work is aimed to design and evaluate the mucoadhesive microspheres of Venlafaxine HCl

In this present work the mucoadhesive microspheres of venlafaxine HCl were prepared employing 2^3factorial design by using Ethyl cellulose (EC) along with Eudragit RS100 and Hydroxy Propyl Methylcellulose (HPMC K4M). In this experimental model, our goal is to determine how the $t_{80\%}$ of drug release and mucoadhesive characters can be affected by adjusting three parameters: concentration of polymers EC, Eudragit RS100 & HPMC K4M of the mucoadhesive microspheres. For each of these parameters, the levels will define for use in this 2-level experiment. In formulations, the low and high levels of EC, EUDRAGIT RS100 and HPMC K4M were 750 mg and 1000 mg, 100 mg, 200 mg and 200 mg, 300 mg respectively.

5.0 PLAN OF WORK

Present work was carried out to design and evaluate the mucoadhesive microspheres of Venlafaxine HCl.

1. **Preformulation studies**
    a) Selection of Mucoadhesive Polymers
    b) Compatibility study
        i. Fourier Transform Infrared Spectroscopy (FTIR)
        ii. Differential Scanning Calorimetry (DSC)

2. **Preparation of standard curve of Venlafaxine HCl**
    a) Standard curved for Venlafaxine HCl in 0.1 N HCl(pH 1.2)
    b) Standard curved for Venlafaxine HCl in phosphate buffer pH 6.8

3. **Formulation of mucoadhesive microspheres of Venlafaxine HCl**

Using a combination of Ethyl Cellulose, and Eudragit RS100 as a rate-controlling polymer along with mucoadhesive polymer HPMC K4M employing 2^3factorial design.

4. Evaluation of the prepared mucoadhesive microspheres of venlafaxine HCl

a) Percentage yield.

b) Particle size distribution by microscopic method.

c) Angle of repose.

d) Bulk density.

e) Drug content.

f) Encapsulation efficiency.

g) Shape and Surface characterization of the prepared micro spheres (SEM).

h) In vivo wash–off test

i) In vitro dissolution studies.

j) Interpretation of drug release mechanism by kinetic models

k) Optimization of the formulation using f_2 similarity factor

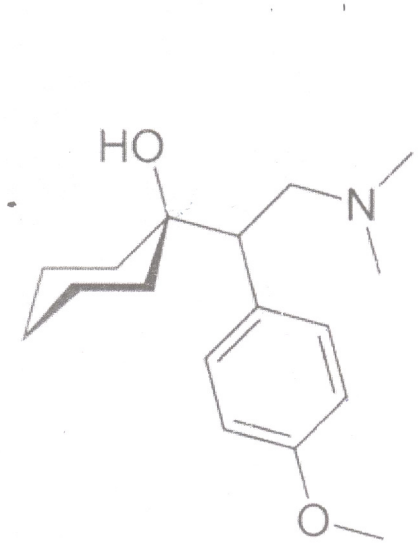
6.0 PROFILES

6.1 DRUG PROFILE [62, 80]

Name of the drug : Venlafaxine HCl

Chemical Name : 1-[2-dimethyl amine-1-(4- methoxy phenyl) Ethyl]cyclohexane-1-ol

Structure :



Molecular Formula	**:**	$C_{17}H_{27}NO_2$ HCl
Molecular Weight	**:**	313.9
Melting point	**:**	216
Category	**:**	Oral antidepressant
Description	**:**	It is crystalline white powder,
Solubility	**:**	Freely soluble in water, methanol, Chloroform &sparingly soluble in acetone
Dose	**:**	37.5 -225mg per day after meals

Clinical pharmacology

Pharmacodynamics :

The mechanism of the antidepressant action of venlafaxine in human is believed to be associated withits potentiation of neurotransmitter activity in the CNS. Preclinical studieshave shown that venlafaxine and its active metabolite, O- desmethyl Venlafaxine(ODV),are potent inhibitors of neuronal serotonin and nor epinephrine reuptake and weak inhibitors of dopamine reuptake. Venlafaxine and ODV have no significant affinity for muscarinic cholinergic, H1 histaminergic, or α1-adrenergic receptorsin vitro. Pharmacological activity at these receptors is hypothesized to be associated with various anticholinergic, sedative, and cardiovascular effects seen with other psychotropic drugs. Venlafaxine and ODV do not posses monoamine oxidase (MAO) inhibitory activity.

Pharmacokinetics

Steady state concentration of venlafaxine and ODV in plasma is attained within 3 days of oral multiple dose therapy .Venlafaxine and ODV exhibited linear kinetics over the dose range of 75 to 225mg/day. mean± SD steady state plasma clearance of venlafaxine and ODV is 1.3± 0.6and0.4±0.2L/hkg ,respectively and (apparent)steady state volume of distribution is 7.5±3.7 and 5.7±1.8L/kg,respectively .Venlafaxine and ODV are minimally bound at therapeutic concentrations to plasma proteins (27% and 30%,respectively)

Mechanism of action

Venlaflaxine HCl is a bicyclic antidepressant, and is usually categorized as a serotoninor epinephrine reuptake inhibitor, but it has been referred to as a serotonium-nor epinephrine –dopamine reuptake inhibitor. It works by blocking the transporter "reuptake" proteins for key neurotransmitters affecting mood, thereby leaving more

active synapse.At low dosage, Venlafaxine blocks serotonium reuptake alone, similarly to a selective serotonium reuptake inhibitor (SSRI). At medium dosages, Venlafaxine blocks the reuptake of nor epinephrine as well as serotonium. At dosages above 300mg/day,it blocks dopamine reuptake in addition to serotonium and nor epinephrine.

Absorption

Venlafaxine is well absorbed and extensively metabolized in the liver. O-desmethylvenlafaxine (ODV) is the only major active metabolite. On the basis of mass balance studies, at least 92% of a single oral dose of Venlafaxine is absorbed. The absolute bioavailability of Venlafaxine is about 45%.

Metabolism and Excretion

Following the absorption, Venlafaxine undergoes the extensive presystemic metabolism in the liver, primarily to ODV ,But also to N-desmethylvenlafaxine, N,O-desmethylvenlafaxine and other minor metabolites. In vitro studies indicate that formation of ODV is catalyzed by CYP2D6;this has been confirmed in a clinical study showing the patients with low CYP2D6 levels had increased levels of Venlafaxine and reduced levels of ODV, compared to people with normal CYP2D6.The differences between CYP2D6 poor and extensive metabolizers are not expected to be clinically important because the sum of venlafaxine and ODV is similar in two groups and venlafaxine and ODV are pharmacologically approximately equiactive and equipotent.

Dosing

The dose of Venlafaxine will be different for different patients. Follow your doctor’s orders or the directions for on the label. The following information includes

only the average doses of Venlafaxine HCl. The number of capsules or tables that you take depends on the strength of the medicine. Also, the number of doses you take each day, the time allowed between doses, and the length of time you take the medicine depend on your special needs.

Therapeutic uses

For mental depression:

For oral extended-release capsule dosage form:

Adults-At first, 75 milligrams(mg) a day, taken in one dose in the morning or evening. However the dose is usually not more than 225mg a day.

Children's-Use and dosage must be determined by your doctor.

For oral tablet dosage form.

Adults, at first, a total of 75mg per day, taken in the smaller doses two or three times during the day. However the dose is usually not more than 375 mg a day.

Children up to 18yrs of age – Use and dose must be determined by your doctor.

For anxiety:

For oral extended-release capsule dosage form:

Adults—At first, 75mg a day taken in one dose in the morning or evening. However the dose is usually not more than 225mg per day.

Children—Use and dose must be determined by your doctor. Take the missed dose as soon as you remember. However if it is almost time for your next regular scheduled dose, skip the missed dose and take only the next one as directed. Do not take double dose for this medication.

Missed dose

Take the missed dose as soon as you remember. However if it is almost time for your next regularly scheduled dose, skip the missed dose and take only the next one as directed. Do not take the double dose for this medication.

Side effect:

Along with its needed effects, a medicine may cause some unwanted effects although not all of these side effects may occur.

More Common: High Blood pressure.

Other side effects may occur that usually do not need medical attention. These side effects may go away during treatment as your body adjusts to the medicine. More common Abnormaldreams; anxietyornervousness; chills; constipation;diarrhea;dizziness; drowsiness; dryness of mouth; heartburn; increased sweating ;loss of appetite; nausea; stuffy; burning; or prickly sensation; trembling; trouble in sleeping; unusual tiredness; weakness; vomiting; weight loss.

Less common: Change in sense of taste muscle tension.

Rare: Convulsions; itching or skin rash; lightheadness or fainting, especially when getting up suddenly from sitting or lying position; lock jaw; menstrual changes; problems in urinating or in urinating or in holding urine; swelling; talking ,feelings and acting with excitement and activity you cannot control trouble in breathing.

After you stop using this medicine your body may need time to adjust. The length of time takes depends on the amount of medicine you were using and how long it you used it. During this period of time check with your doctor if you notice any of the following side effects:

Changes in dreaming; dizziness; dryness; headache; increased sweating; nausea; nervousness; trouble in sleeping ; unusual tiredness or weakness.

Storage

- Keep out of reach of children.
- Store away from heat and direct light
- Do not store in the bathroom near the kitchen sink, or in the other damp places. Heat or moisture may cause the medicine to break down.
- Do not keep outdated medicine or medicine no longer needed .Be sure that any discarded medicine is out of reach of children.

6.2. EXCIPIENTS PROFILE

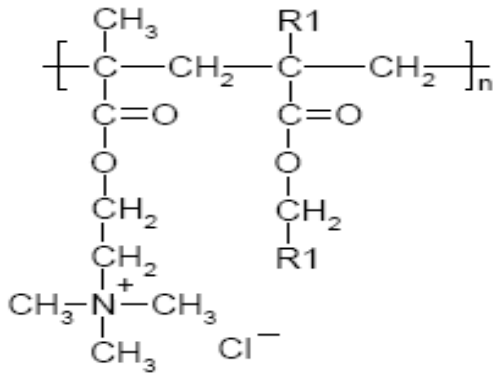
6.2.1 Eudragit (Polymethacrylates) [63]

Polymethacrylates are synthetic cationic and anionic polymers of dimethylaminoethyl methacrylates, methacrylic acid, and methacrylic acid esters in varying ratio.

Chemical Name **:** Poly(ethyl acrylate, methyl methacrylate, Trimethylammonioethyl methacrylate chloride)

1: 2: 0.1

Structure :



R = H or CH_3

For Eudragit –RS100

R^1 = CH_3, C_2H_5

Molecular Weight : ≥ 100,000 Daltons

Synonym : Acryl-EZE; Acryl-EZE MP; Kolicoat MAE 30 D; Kollicoat MAE 30 DP; polymeric methacrylates.

Trade Name : Eudragit RS 100

Solubility : Soluble in Acetone and alcohols, Dichloromethane, Solvent Ethyl acetate. Insoluble in water and Petroleum ether,

Description : Eudragit RS 100 are crystalline, white powders with a

slight amine-like odor.

Density (Bulk) : 0.40 g/cm^3

(Tapped) : 0.424 g/cm^3

(True) : 0.816.0.836 g/cm^3

Viscosity	**:**	≤ 7-100 m Pa s
Stability and Storage Condition	**:**	Dry crystalline polymer forms are stable at temperature less than 30°C. Above this temperature, powders tend to form clumps, although this does not affect the quality of the substance and the clumps can readily be broken up. Dry powders are stable for at least 3 years if stored in a tightly closed container at less than 30°C.
Safety	**:**	Polymethacrylate copolymers are widely used as Film coating materials in oral pharmaceutical formulations. They are also used in topical formulations and are generally regarded as nontoxic and nonirritant materials.
Handling Precautions	**:**	Eye protection, gloves, and a dust mask or Respirator are recommended. Polymethacrylates should be handled in well-ventilated environment and measures should be taken to prevent dust formation.
Applications	**:**	Polymethacrylates are primarily used in

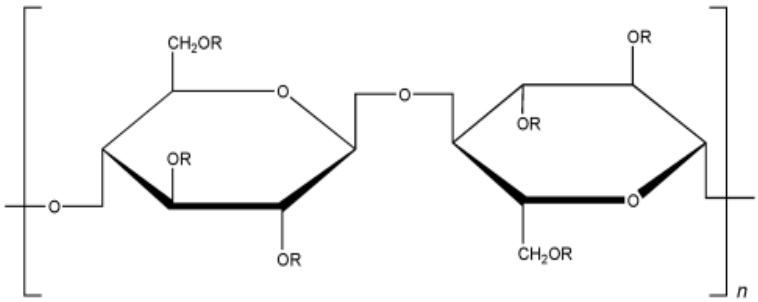
microencapulation, oral capsule and tablet formulations as film-coating, Enteric coating agent and for sustained release.

6.3. HYDROXYPROPLY METHYL CELLULOSE [64]

It is mixed alkyl-hydroxy alkyl cellulose ether and may be regarded as regarded as the propylene glycol ether of methyl cellulose.

Chemical name **:** Cellulose, 2- hydroxypropyl methylether.

Structure



where R is H, CH_3, or $CH_3CH(OH)CH_2$

Molecular weight **:** 10000–1500000 daltons

Synonym **:** Hypromellose

Trade name **:** Methocel

Solubility **:** Methocel soluble in cold water, forming a viscous colloidal solution; practically insoluble in chloroform, ethanol 95%, and ether, but soluble in mixtures of ethanol and dichloro methane, mixtures of methanol and dichloromethane,and mixtures of water and alcohol.

Description **:** An odorless and tasteless, white or creamy-white fibrous or granular powder.

Density **:** Bulk : 0.341 g/cm^3

: Tapped : 0.557 g/cm^3

: True : 1.326 g/cm^3

Specific gravity **:** 1.26 g/cm^3

Viscosity **:** HPMC K4M, 4000 mPa s ; (2% (w/v) aqueous solution)

Grade **:** K4M

pH **:** 5.5–8.0 for a 1% w/w aqueous solution.

Stability **:** Very stable in dry conditions. Solutions are stable at pH 3-11

Applications **:** 1)In oral products, hypromellose is primarily used as a tablet Binder, in film-coating, and as a matrix for use in extended release tablet formulations.

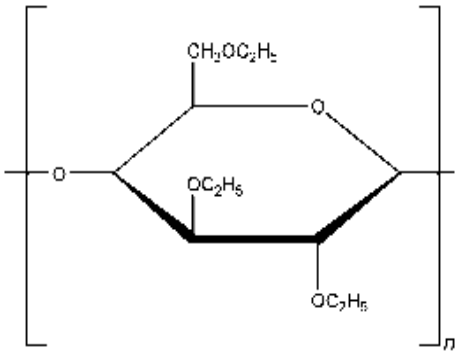
2) Concentrations between 2% and 5% w/w may be used as a binder in either wet- or dry-granulation processes.

3) High-viscosity grades may be used to retard the release of drugs from a matrix at levels of 10–80% w/w in tablets and capsules.

6.4. ETHYL CELLULOSE[65]

Chemical name **:** Cellulose ethyl ether.

Structure :



Functional Category : Coating agent; flavoring fixative; tablet binder; tablet filler; Viscosity - increasing agent.

Solubility : Soluble in chloroform, ethanol (95%), ethyl acetate, methanol, and toluene. Practically insoluble in glycerin, propylene glycol, and water.

Description : Ethylcellulose is a tasteless, free-flowing, white to light tan colored powder.

Bulk density : 0.4 g/cm^3

Viscosity : 7 to 100 mPa s for a 5% w/v solutions

Specific gravity : 1.12–1.15 g/cm^3

Stability : Ethylcellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic

materials than are cellulose esters.

Incompatibility : Incompatible with paraffin wax and microcrystalline wax.

Safety : Ethylcellulose is widely used in oral and topical Pharmaceutical formulations. It is also used in food products.

Application : 1) The main use of ethylcellulose in oral formulations is as a hydrophobic coating agent for tablets and granules.

2) Ethylcellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of a formulation; for example, where granules are coated with ethylcellulose to inhibit oxidation.

3) Modified-release tablet formulations may also be produced using ethylcellulose as a matrix former.

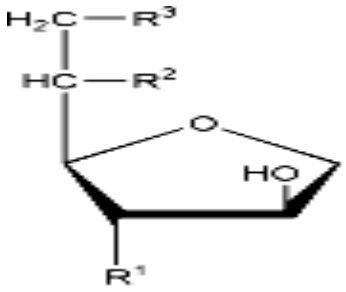
6.5 SPAN-80

Synonyms: Sorbitan Monolaurate

Empirical Formula: $C_{18}H_{34}O_6$ (Sorbitan Monooleate)

Molecular Weight: 346

Structural formula:



$R^1 = R^2 = OH$, $R^3 = R$ for sorbitan monoesters

where R = $(C_{11}H_{23})COO$ for laurate

Functional category: Emulsifying agent; nonionic surfactant; solubilizing agent; wetting and dispersing/suspending agent.

Application in pharmaceutical formulation:

- Span 80 are widely used in cosmetic, food products, and pharmaceutical formulations as lipophilic non-ionic surfactants.
- They are mainly used in pharmaceutical formulations as emulsifying agent in the preparation of creams, emulsion, and ointments for topical application.
- When used alone span 80 produce stable water –in-oil emulsion and micro emulsion.

Description: Sorbitan esters occur as cream to amber-colored liquids or solid with a distinctive odor and taste. Span 80 occurs as yellow viscous liquid

.

Typical properties:

POLYSORBATE	Density (g/cm^3)	HLB VALUE	Viscosity
Span 80	1.01	8.6	2970-4080

Stability and storage condition:

Gradual soap formation occurs with strong acid or bases; sorbitan esters are stable in weak acid or bases. Sorbitan esters should be stored in a well- closed container in a cool, dry place.

Safety:

They are generally regarded as non-toxic and non-irritant materials. However, there have been occasional reports of hypersensitive skin reaction following the topical application of product containing sorbitan esters. When heated to decomposition the sorbitan esters emit acrid smoke and irritating fumes.

7.0 METHODOLOGY

7.1 MATERIALS USED

S.No.	MATERIALS	SUPPLIED BY
1.	Venlafaxine HCl	ORCHID Pharma Ltd., Kanchipuram
2.	Ethyl Cellulose (30-50 cps)	Himedia Laboratories Ltd. Mumbai
3.	Hydroxy Propyl MethylCellulose	Orchid Lab, Chennai.
4.	Eudragit RS100	Micro Labs, Hosur.
5.	Span 80	Loba Chemical. Pvt. Ltd. Mumbai
6.	Liquid paraffin (light)	Loba Chemical. Pvt. Ltd, Mumbai
7.	Acetone AR	Research labs fine chemicals, Mumbai.
8.	Concentrated HCl LR	Nice Chemicals Pvt. Ltd, Chennai
9.	Potassium dihydrogen Phosphate AR	Nice Chemicals Pvt. Ltd, Cochin
10.	Sodium Hydroxide LR	Nice Chemicals Pvt. Ltd, Cochin
11.	Whatmann filter Paper-No.44	Research labs fine chemicals, Mumbai
12.	Petroleum ether	Chempure, Chennai

7.2. EQUIPMENTS USED

S.No.	EQUIPMENTS	SUPPLIED BY
1.	Magnetic Stirrer	Elektrocrafts Pvt.Ltd., Mumbai

2.	Microscope	Acculab
3.	UV/Visible spectrophotometer Double beam	Perkin Elmer Lambda25 UV/VIS Spectrophotometer
4.	Dissolution Test Apparatus- U.S.P. Standards	Veego India pvt Ltd.
5.	Single Pan Digital Balance	Shimadzu. ELB 300
6.	Hot Air Oven	Inlab equipments
7.	Digital pH meter	Hanna Instrument.
8.	FTIR Spectrophotometer	Perkin Elmer Spectrum- RX1 FTIR - Spectrophotometer
9.	Scaning Electron Microscope (SEM)	Joel model JSM 6400,Tokyo
10.	Differential scanning calorimetry	DSC DA 2010 Shimadzu, Japan

7.3. PREFORMULATION STUDIES

Before formulation of drug substances into a dosage form, it is essential that drug and polymer should be chemically and physically characterized. Preformulation studies give the information need to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the fabrication of a dosage form.

7.3.1. SELECTION OF MUCOADHESIVE MICROSPHERES

Mucoadhesive polymers are water-soluble and water insoluble polymers, which are swellable networks, joined by cross-linking agents. The polymers should posse's optimal polarity to make sure it is sufficiently wetted by the mucus and optimal fluidity that permits the mutual adsorption and inter penetration of polymer and mucus to take place. An ideal polymer for a mucoadhesive drug delivery system should have the following characteristics.

1. The polymer and its degradation products should be nontoxic and non absorbance from the gastrointestinal tract.
2. It should be a nonirritant to the mucous membranes.
3. It should preferably form a strong non-covalent bond with the mucin epithelium cell surfaces.
4. It should adhere quickly to moist tissue and should possess some site specificity.
5. It should allow easy incorporation of the drug and offer no hindrance to its release.
6. The polymer must not decompose on storage or during shelf life of the dosage form. In the present work Eudragit RS100 and Hydroxy propyl methylcellulose K4M were chosen.

7.3.2. COMPATIBILITY STUDIES

One of the requirements for the selection of suitable excipients or carrier for pharmaceutical formulation is its compatibility. Therefore in the present work a study was carried out by using FTIR spectrophotometer and Differential Scanning Calorimeter

(DSC) to find out if there is any possible chemical interaction between Venlafaxine HCl, Ethyl Cellulose, Hydroxy Propyl Methyl Cellulose and Eudragit RS100.

Pre formulation studies for drug and carrier interaction

a) Fourier Transform Infrared Spectrophotometry (FTIR) [66,79]

Compatibility study of drug with the excipients was determined by FTIR Spectroscopy using : Perkin Elmer Spectrum- RX1 FTIR - Spectrophotometer . The pellets were prepared at high compaction pressure by using KBr and the ratio of sample to KBr is 1:100. The pellets thus prepare were examined and the spectra of drug and other ingredients in the formulations were compared with that of the original spectra.

b) Differential scanning colorimeter (DSC)[67]

Differential scanning calorimeter is used to measure the specific heat and enthalpies of transition. When a sample undergoes a thermal transition, the power to the heater is adjusted to maintain the temperature, and a signed proportional to the power difference is plotted on the second axis of the recorder is known as thermogram. The area under the resulting curve is direct measure of the heat of transition. Thermograms were obtained by using a differential scanning colorimeter at a heating rate 10ºC /min over a temperature range of 30to 300°C. The sample was hermetically sealed in an aluminum crucible. Nitrogen gas was purged at the rate of 40 ml/min. For maintaining inert atmospheres.

7.4 CONSTRUCTION OF STANDARD CURVE FOR VENLAFAXINE HCI

Venlafaxine HCl can be estimated spectrophotometrically at 224.0 nm as it obeys Beer-Lambert's law.

Preparation of 0.1 N HCl

Dilute 8.5 ml of concentrated hydrochloric acid with water to produce 1000 ml.

Preparation of standard drug solution

Stock Solution

100 mg of Venlafaxine HCl was dissolved in100 ml of 0.1N HCl, so as to get a stock solution of 1000 µg/ml concentration.

Standard Solution

20 ml of stock solution was made to 100 ml with 0.1N HCl, thus giving a concentration of 200 µg/ml. Aliquot of standard drug solution ranging from 1 to 8 ml were transferred in to 10 ml volumetric flask and were diluted up to the mark with 0.1N HCl. Thus the final concentration ranges from 2-16 µg/ml. Absorbance of each solution was measured at 224.0 nm against 0.1N HCl as a blank. A plot of concentrations of drug versus absorbance was plotted.

The linear regression analysis was done on absorbance data points.

A straight-line equation was generated to facilitate the calculation of amount of drug. The equation is as follows.

$$Y = mx + c$$

Where ***Y***= Absorbance, **m** = slope**, x** = Concentration, ***c*** = Intercept.

Preparation of phosphate buffer p^H 6.8

Place 50.0 ml of 0.2M potassium dihydrigen phosphate in a 20ml volumetric flask, add the specified volume of 22.4ml of 0.2M sodium hydroxide and then add water to make the volume.

0.2Mpotassium dihydrogen phosphate

Dissolve27.218gm of potassium dihydrogen phosphate in distilled water and dilute to 1000ml with distilled water.

0.2Msodium hydroxide solution

Dissolve 8g of sodium hydroxide in distilled water and dilute to 1000ml with distilled water.

Preparation of standard drug solution

Stoke solution

100mg of Venlafaxine HCl was dissolved in 100 ml of phosphate buffer p^H6.8, so as to get a solution of 1000µg/ml concentration.

Standard solution

20ml of stoke solution was made to 100ml with phosphate buffer p^H6.8, thus giving a concentration of 200 µg/ml. Aliquot of standard drug solution ranging from 1 to 8 ml were transferred in to 10 ml volumetric flask and were diluted up to the mark with phosphate buffer p^H6.8. Thus the final concentration ranges from 2-16 µg/ml. Absorbance of each solution was measured at 224.0 nm against phosphate buffer p^H 6.8 as a blank. A plot of concentrations of drug versus absorbance was plotted

Table.5

CALIBRATION CURVE FOR THE ESTIMATION OF VENLAFAXINE HCl IN 0.1 N HCl

Sl. No	**Concentration (µg/ml)**	**Absorbance in** 0.1N HCl

1.	0	0
2.	2	0.044
3.	4	0.092
4.	6	0.134
5.	8	0.169
6.	10	0.206
7.	12	0.260
8.	14	0.304
9.	16	0.332
Slope		0.020966667
Correlation Coefficient		0.998886793

Figure 01

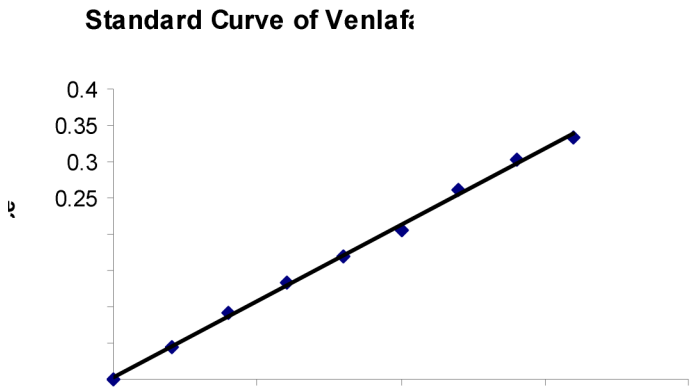


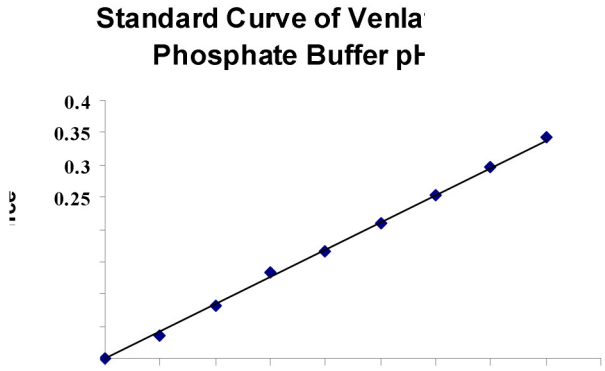
Table.6

CALIBRATION CURVE FOR THE ESTIMATION OF VENLAFAXINE HCL IN PHOSPHATE BUFFER P^{H} 6.8

Sl. No	**Concentration (µg/ml)**	**Absorbance in Phosphate buffer pH 6.8**
1.	0	0
2.	2	0..036
3.	4	0.082
4.	6	0.132
5.	8	0.166
6.	10	0.209
7.	12	0.254
8.	14	0.296

9.	16	0.342
Slope		0.021408333
Correlation Coefficient		0.999626534

Figure 02



7.5 PREPARATIONOFMUCOADHESIVEMICROSPHERES BY EMULSION-SOLVENT EVAPORATION TECHNIQUE

Accurately weighed quantity of the polymer (Ethyl Cellulose & Eudragit RS100) was dissolved in 20 ml of acetone. Weighed quantity of VENLAFAXINE HCl (drug) and mucoadhesive polymer HPMC K4M (previously passed through the sieve # 150) were then dispersed in the above polymer phase; and it was stirred for 2 hours. Then it was emulsified with the 100 ml of liquid paraffin containing 1.0 w/v of Span 80 with continuous stirring at 800rpm under a magnetic stirrer. The stirring was continued for 2 hours to ensure complete evaporation of acetone. The microspheres were then separated from liquid paraffin by filtration through Whatmann filter paper No. 44, washed three times with 50 ml of petroleum ether, and air dried for 12 hours. All the formulations of microspheres were prepared in the same way. The detailed compositions of various formulations prepared employing 2^3 factorial designs were mentioned in Table No.7 and 8

Table. 7

LEVELS OF FACTORS

Factors	Low level	High level
Ethyl cellulose	750	1000
HPMC K4M	200	300
Eudragit RS100	100	200

Table. 8

COMPOSITION OF FORMULATIONS OF MUCOADHESIVE MICROSPHERES OF VENLAFAXINE HCL

Formulation Code	Venlafaxine Hcl (mg)	EC (mg)	Eudragit RS100 (mg)	HPMC K4M (mg)
F_1	500	750	100	200
F_2	500	1000	100	200
F_3	500	750	200	200
F_4	500	1000	200	200
F_5	500	750	100	300
F_6	500	1000	100	300
F_7	500	750	200	300
F_8	500	1000	200	300

7.6 EVALUATION OF THE PREPARED MUCOADHESIVE MICROSPHERES

7.6.1. DETERMINATION OF PERCENTAGE YIELD OF MICROSPHERES [68]

Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formula given below.

$$\% \text{ yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer}} \text{ x } 100$$

7.6.2. MICROSPHERE SIZE ANALYSIS[69]

Microsphere size distribution

Microsphere size determination was done by optical microscopy method. Size distribution plays a very important role in determining the release characteristics of the microspheres.

7.6.3. ANGLE OF REPOSE[70]

The fractional force in the loose powder can be measured by the angle of repose. This is the maximum angle possible between the surfaces of the site of the powder to the horizontal plane.

Angle of repose was calculated by static method using funnel. Funnel was kept on triangular stand, which was kept on horizontal plane. The sample was introduced into

the funnel, as the pile forms; it reaches to the tip of funnel. The diameter of the pile was noted. The angle of repose (θ) is calculated by the following formula,

$$\theta = \tan^{-1} (h/r)$$

Where,

h = pile height of microspheres;

r = radius of the circular are formed by the microspheres on the ground.

7.6.4 DETERMINATION OF BULK DENSITY [71]

The bulk density was determined by 3-tap method. Bulk density is defined as, "the mass of powder divided by the bulk volume". The packing characteristics of the powder play an important role in determining physical properties of product. According to the standard procedure for obtaining bulk density, weighed quantities of prepared microspheres were filled in 10 ml of graduated cylinder the initial volume was noted. After tapping for three times the final volume was noted. The bulk density was calculated as per following formula:

$$\rho = \frac{W_O}{V_O}$$

Where,

ρ= Bulk density

Wo =Weight of sample in gm

Vo= Final volume after tapping

7.6.5. DETERMINATION OF DRUG CONTENT [72]

Accurately weighed 100 mg microspheres, crushed in glass mortar and pestle and the powdered microspheres were suspended in 100 ml of 0.1N HCl. After 12 hours the solution was filtered and the filtrate was analyzed for the drug content using UV –Visible spectrophotometer at 224nm.

7.6.6 ENCAPSULATION EFFICIENCY[73]

Encapsulation efficiency was calculated using the following formula;

$$\text{Encapsulation efficiency} = \left(\frac{\text{Estimated drug content \%}}{\text{Theoretical drug content \%}} \text{x} 100 \right)$$

Where,

Wo = initial weight of the dry microspheres,

We = weight of the swollen microspheres at equilibrium swelling in the media.

7.6.7. IN VIVO WASH-OFF TEST[74]

The mucoadhesive property of microspheres was evaluated by an *In vitro* adhesion testing method known as wash-off method**.** Freshly excised piece of intestinal mucosa (2 x 2 cm) from goat were mounted on to glass slides (3 x 1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support, about 25 microspheres were spread on to each wet rinsed tissue specimen and immediately thereafter the support was hung on to the arm of a USP tablet disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was

given slow, regular up-and-down moment in the test fluid (900 ml of 0.1N HCl/phosphate buffer pH 6.8 at 37 ± 0.5°C. At the end of one hour, and at the hourly intervals up to 5 hours, the machine was stopped and number of microspheres still adhering to tissue was calculated. The studies were carried out in triplicate.

Figure. 3

***IN VITRO* WASH-OFF TEST FOR MUCOADHESION**

7.6.9 IN-VIVO SISSOLUTION STUDIES[76]

Dissolution studies were carried out for all the formulations, employing USP XXIII apparatus (Basket method) at 37 $\pm$ 0.5°C rotated at constant speed of 50 rpm using 0.1N HCl as the dissolution medium for first 2 hrs and remaining in phosphate buffer pH 6.8. A sample of microspheres equivalent weight to 75 mg of venlafaxine HCl was used in each test. An aliquot of the sample was periodically with drawn at suitable time interval and the volumes were replaced with fresh dissolution medium in order to maintain the sink condition. The sample was analyzed spectrophotometrically at 224nm.

7.6.10. KENETICS OF DRUG RELEASE [77, 81, 82]

In order to understand the mechanism and kinetic of drug release, the drug release data of the *in-vitro* dissolution study were analyzed with various kinetic model like zero order, first order, Higuchi's, Peppa's and Coefficient of correlation (r) values were calculated for the linear curves by regression analysis of the above plots.

7.6.11. SHAPE AND SURFASE CHARECTERISATION[78]

The shape and surface characterization of microspheres were observed under a Scanning Electron Microscope (SEM). The instrument used for this study was Joel model JSM 6400, Tokyo Scanning Electron Microscope. The microspheres were mounted directly on to the SEM sample stub, using double-sided sticking tape, and

coated with gold film (thickness 200 nm) under reduced pressure (0.001 torr) and photographed.

8.0 RESULTS

8.1 PRE FORMULATION STUDIES FOR DRUG AND CARRIER INTERACTION

a) Fourier Transform Infrared Spectrophotometry (FTIR)

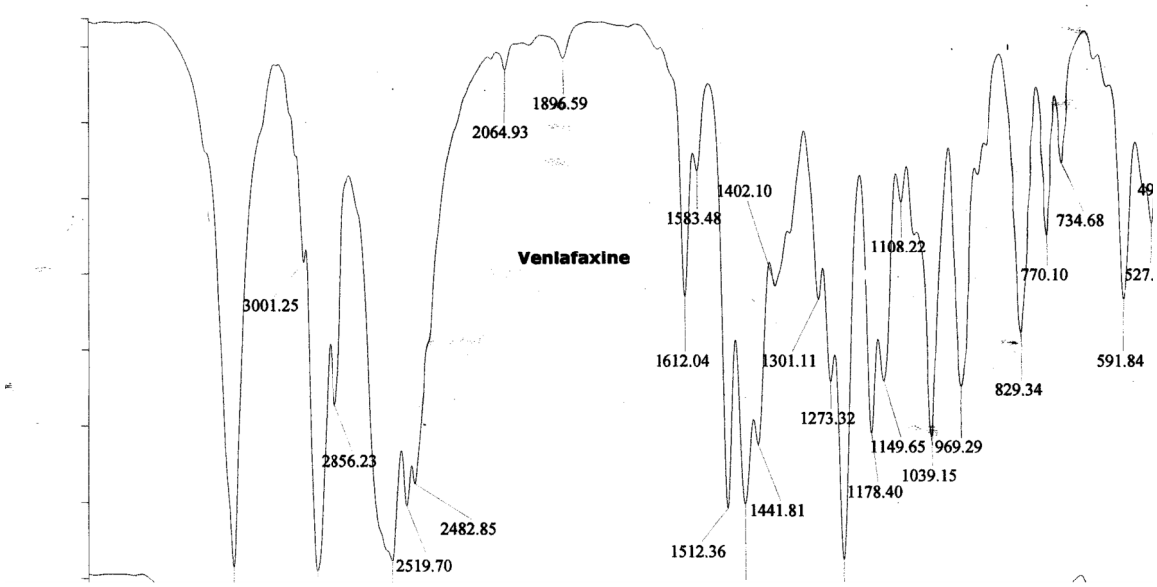
Infrared spectra for pure Venlafaxine HCl and for the physical mixture of Venlafaxine HCl and all the polymers were determined to check the intactness of the drug in the polymer mixture using Perkin Elmer Spectrum- RX1 FTIR - Spectrophotometer by disc method. The following table shows the wave number for the characteristic bands in the IR spectra of pure Venlafaxine HCl

Table. 9

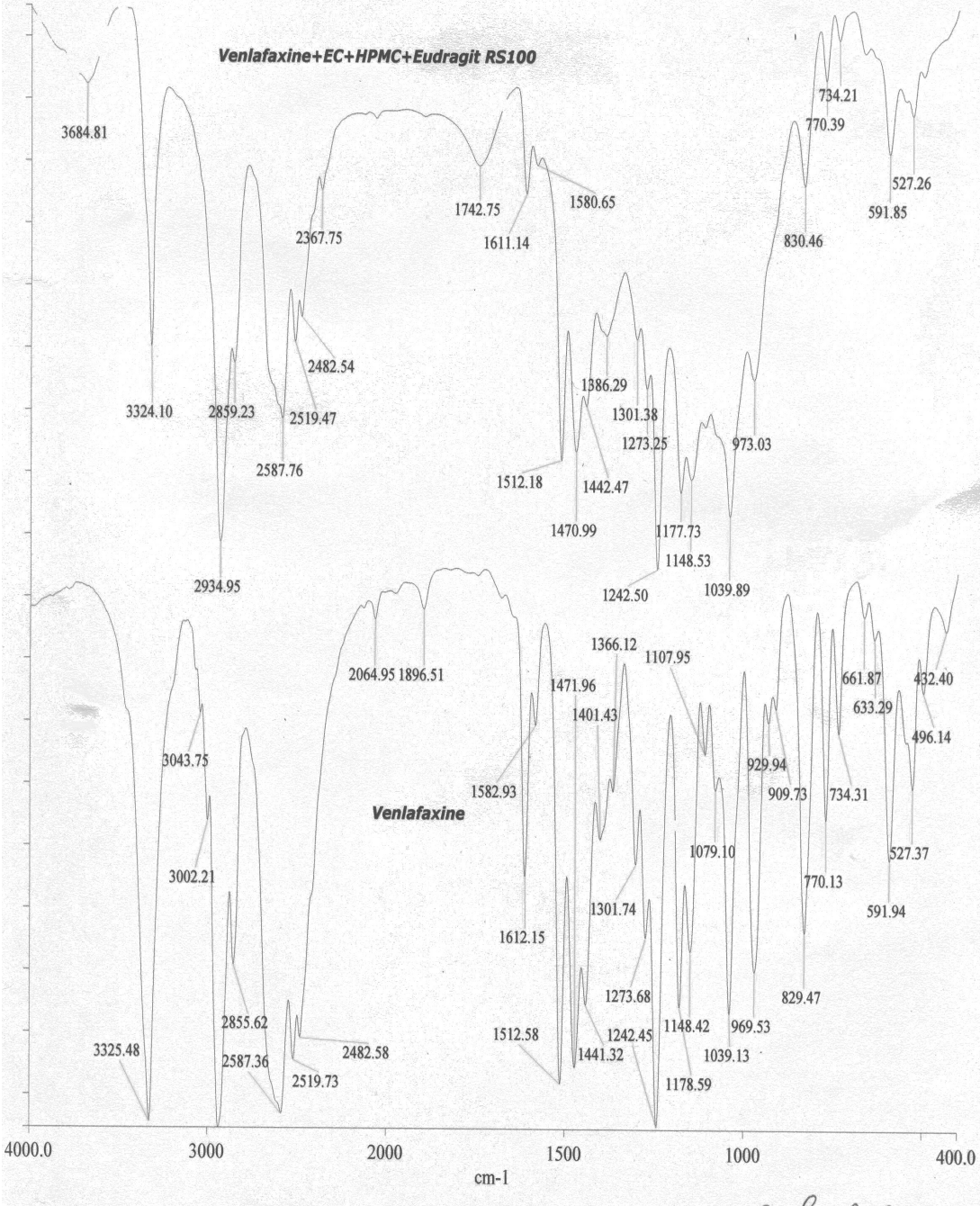
Wave number in cm^{-1}	Characteristic bands
2938	Alkane/cyclohexane
1470	Alkane(C-H bending)
829	Aromatic ring
1612	Tertiary amine
3325	O-H stretching
1108,1149	Tertiary amine
1039	Tertiary alcohol

FTIR spectra for pure drug, for the carriers and for the physical mixture of both are shown in **Figure 4, 5**

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY
TIRUCHENGODE



SWAMY VIVEKANANDHA COLLEGE OF PHARMACY
TIRUCHENGODE



ANALYST

b) Differential scanning colorimeter (DSC)

DSC thermogram of venlafaxine HCl and physical mixture of drug and polymers are shown in **Figure. 6 to 7**. DSC thermogram of pure drug has shown a melting endotherm at 216.6°C with normalized energy. The thermogram of physical mixture showed that the venlafaxine HCl melting onset temperature decreased to 213.4° C because of the presence of polymers in the physical mixture.

Figure. 6

DSC Spectra for pure drug of venlafaxine Hcl

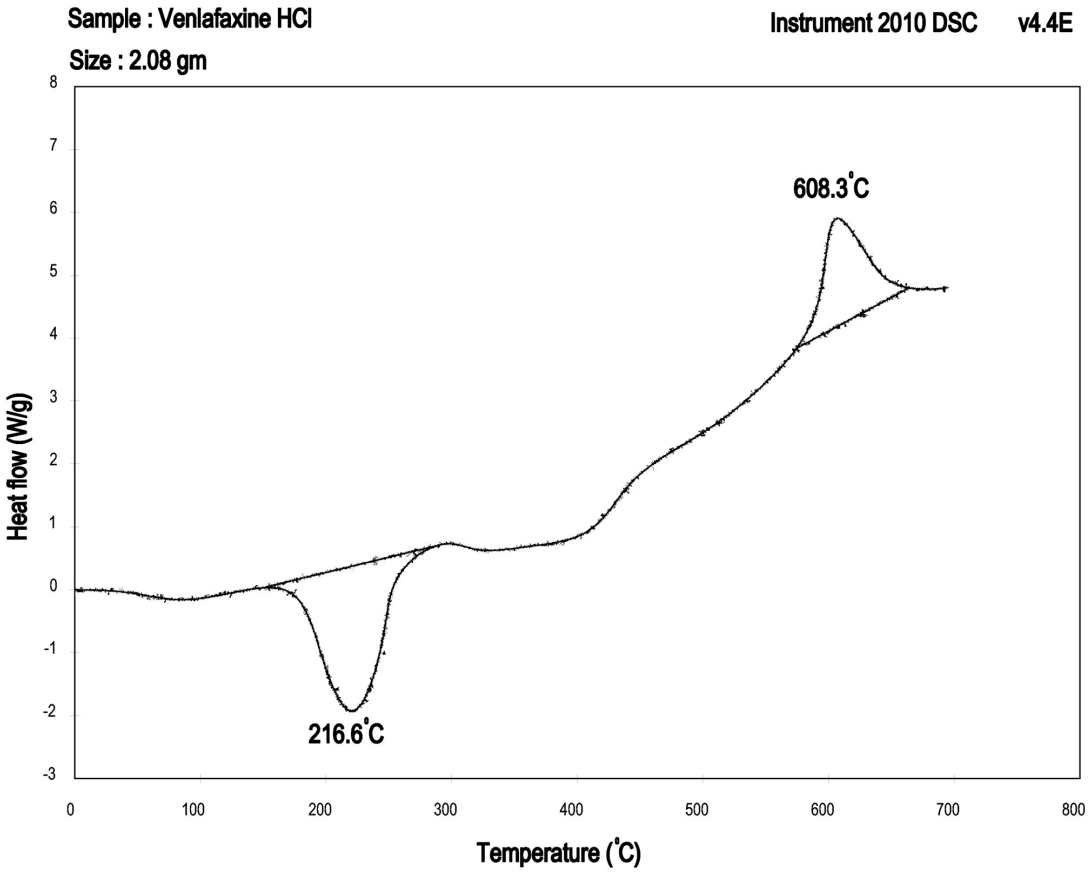


Figure. 7

DSC Spectra for Physical mixture of VenlafaxineHcl + Eudragit RS100+ HPMC K4M+EC

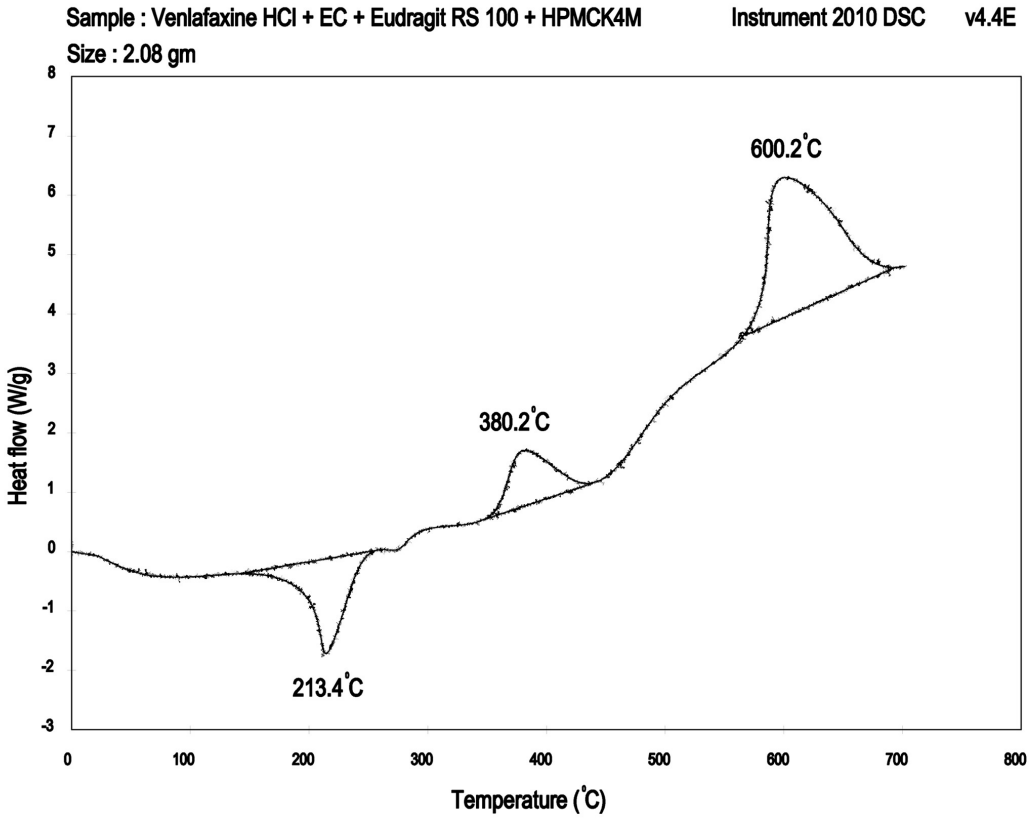


Table. 10

DATA FOR PERCENTAGE YIELD OF FORMULATIONS OF MUCOADHESIVE MICROSPHERES OF VENLAFAXINE HCL

Formulation code	Percentage yield (%)
F_1	47.10
F_2	80.00
F_3	76.91
F_4	43.68
F_5	91.82
F_6	48.32
F_7	62.06
F_8	87.00

Table. 11

SIZE DISTRIBUTION ANALYSIS OF MICROSPHERES FOR F1

Size range	Mid point	Calibration factor Particle size in μm (x)	Number of particle (n)	n*x	Average size in μm	
11-12	11.5	3.33	38.30	5	191.5	65.66
13-14	13.5	3.33	44.96	6	269.8	
15-16	15.5	3.33	51.62	12	619.44	
17-18	17.5	3.33	58.28	10	582.75	
19-20	19.5	3.33	64.94	19	1233.76	
21-22	21.5	3.33	71.60	21 1503.50		
23-24	23.5	3.33	78.26	19	1486.85	
25-26	25.5	3.33	84.92	8	679.32	
27-28	27.5	3.33	91.58	0	0	
				∑n= 100	∑nx=6566.01	

Table. 12

SIZE DISTRIBUTION ANALYSIS OF MICROSPHERES FOR F2

Size range	Mid point	Calibration factor Particle size in μm (x)	Number of particle (n)	n*x	Average size in μm	
13-14	13.5	3.33	44.955	0	0	71.13
15-16	15.5	3.33	51.615	2	103.23	
17-18	17.5	3.33	58.275	8	466.20	
19-20	19.5	3.33	64.935	25	1623.20	
21-22	21.5	3.33	71.595	40	2863.80	
23-34	23.5	3.33	78.255	15 1173.83		
25-26	25.5	3.33	84.915	5	424.58	
27-28	27.5	3.33	91.58	5	457.88	
				∑n= 100	∑nx=7112.88	

Table 13

SIZE DISTRIBUTION ANALYSIS OF MICROSPHERES FOR F3

Size range	Mid point	Calibration factor	Particle size in μm (x) Number of particle (n)	n*x	Average size in μm	
15-16	15.5	3.33	51.615	0	0	
17-18	17.5	3.33	58.275	6	349.65	
19-20	19.5	3.33	64.935	20	1298.70	
21-22	21.5	3.33	71.595	50	3570.75	72.17
23-34	23.5	3.33	78.255	11	860.81	
25-26	25.5	3.33	84.915	8	679.32	
27-28	27.5	3.33	91.58	5	457.88	
				∑n= 100	∑nx=72171	

Table 14

SIZE DISTRIBUTION ANALYSIS OF MICROSPHERES FOR F4

Size range	Mid point	Calibration factor	Particle size in μm (x)	Number of particle (n)	n*x	Average size in μm
15-16	15.5	3.33	51.615	0	0	78.59
17-18	17.5	3.33	58.275	4	233.1	
19-20	19.5	3.33	64.935	9	584.42	
21-22	21.5	3.33	71.595	15	1073.93	
23-34	23.5	3.33	78.255	35	2738.93	
25-26	25.5	3.33	84.915	24	2037.96	
27-28	27.5	3.33	91.58	13	1190.48	
				∑n= 100 ∑nx=7858 .8		

Table 15

SIZE DISTRIBUTION ANALYSIS OF MICROSPHERES FOR F5

Size range	Mid point	Calibration	Particle size in μm	Number of	N*x	Average size in μm

		factor	(x)	particle (n)		
15-16	15.5 3.33	51.615	0	0	81.92	
17-18	17.5	3.33	58.275	0	0	
19-20	19.5	3.33	64.935	7	454.55	
21-22	21.5	3.33	71.595	10	715.95	
23-34	23.5	3.33	78.255	36	2817.18	
25-26	25.5	3.33	84.915	20	1698.30	
27-28	27.5	3.33	91.575	22	2014.65	
29-30	29.5	3.33	98.24	05	493.18	
				∑n= 100	∑nx=8192.0	

Table 16

SIZE DISTRIBUTION ANALYSIS OF MICROSPHERES FOR F6

Size range	Mid point	Calibration factor	Particle size in μm (x)	Number of particle (n)	N*x	Average size in μm
15-16	15.5	3.33	51.615	0	0	83.76
17-18	17.5	3.33 58.275	0	0		
19-20	19.5	3.33	64.935	4	259.74	

21-22	21.5	3.33	71.595	9	644.36
23-34	23.5	3.33	78.255	29	2269.40
25-26	25.5	3.33	84.915	22	1868.13
27-28	27.5	3.33	91.575	30	2747.25
29-30	29.5	3.33	98.24	6	589.41
				∑n= 100	∑nx=8376.2

Table. 17

SIZE DISTRIBUTION ANALYSIS OF MICROSPHERES FOR F7

Size range	**Mid point**	**Calibration factor**	**Particle size in μm (x)**	**Number of particle (n)**	**n*x**	**Average size in μm**
21-22	21.5	3.33	71.595	10 715.95	100.04	
23-34	23.5	3.33	78.255	4	313.02	
25-26	25.5	3.33	84.915	5	425.575	
27-28	27.5	3.33	91.575	15	1373.625	
29-30	29.5	3.33	98.235	11	1080.585	
31-32	31.5	3.33	104.895	33	3461.535	
33-34	33.5	3.33	111.555	5	557.775	
35-36	35.5	3.33	118.215	7	827.505	
37-38	37.5	3.33	124.875	10	1248.75	
				∑n= 100	∑nx=10004.26	

Table. 18

SIZE DISTRIBUTION ANALYSIS OF MICROSPHERES FOR F8

Size range	Mid point	Calibration factor	Particle size in μm (x)	Number of particle (n)	n*x	Average size in μm
21-22	21.5	3.33	71.595	2	143.19	105.43
23-34	3.33		5	391.275		
23.5		78.255				
25-26	25.5	3.33	84.915	2	169.83	
27-28	27.5	3.33	91.575	11	1007.325	
29-30	29.5	3.33	98.235	12	1178.82	
31-32	31.5	3.33	104.895	31	3251.745	
33-34	33.5	3.33	111.555	16	1784.88	
35-36	35.5	3.33	118.215	7	827.505	
37-38	37.5	3.33	124.875	10	1248.75	
39-40	39.5	3.33	131.535	4	526.14	
				∑n= 100	∑nx=10529.48	

Table 19

THE ARITHMETIC MEAN SIZE ANALYSIS OF MICRO SPHERES

OF VENLAFAXINE HCL

Formula code	Average particle size in µm
F1	65.66
F2	71.13
F3	72.17
F4	78.59
F5	81.92
F6	83.76
F7	100.04
F8	105.30

Table. 20

DATA FOR ANGLE OF REPOSE OF FORMULATIONS OF

MUCOADHESIVE MICROSPHERES OF VENLAFAXINE HCL

Formula code	**Angle of repose** **$\theta=\tan^{-1}(h/r)$ Mean ± S.D** ***(n=3)***
F_1	26° 21' ± 0.958
F_2	24° 22' ± 1.305
F_3	25° 20' ± 1.572
F_4	24° 10' ± 2.469
F_5 22° 53' ± 2.258	
F_6	23° 25' ± 0.989
F_7	22° 12' ± 1.801
F_8	25° 11' ± 1.878

Table. 21

DATA FOR BULK DENSITY OF FORMULATIONS OF

MUCOADHESIVE MICROSPHERES OF VENLAFAXINE HCL

Formula code	Bulk Density gm/cm^3 ± SD
F_1	0.551 ± 0.009
F_2	0.561 ± 0.009
F_3	0.572 ± 0.016
F_4	0.595 ± 0.027
F_5	0.531 ± 0.008
F_6	0.620 ± 0.029
F_7	0.501 ± 0.013
F_8	0.501 ± 0.013

Table. 22

DATA FOR PERCENTAGE DRUG CONTENT OF FORMULATIONS OF MUCOADHESIVE MICROSPHERES OF VENLAFAXINE HCL

Formula code	Percentage Drug Content (mean % ± SD)
F_1	22.74± 0.060
F_2	24.71± 0.540
F_3	24.65± 1.406
F_4	18.00± 0.040
F_5	28.20± 0.045

F_6	19.47± 0.050
F_7	25.67± 0.04
F_8	73.51± 0.050

Table. 23

DATA FOR PERCENT ENTRAPMENT EFFICIENCY OF FORMULATIONS

MUCOADHESIVE MICROSPHERES OF VENLAFAXINE HCL

Formula code	**Theoretical drug content in %**	**Practical drug content in %**	**Entrapment Efficiency in %**
F_1	32.26	22.74	70.49
F_2	27.78	24.71	88.94
F_3	30.30	24.65	81.35
F_4	26.31	18.00	68.38
F_5	30.30	28.20	93.08
F_6	26.32	19.47	73.97
F_7	28.57	25.67	89.86
F_8	25.00	18.38	73.51

Table 24

DATA FOR *IN VITRO* WASH-OFF TEST FOR MUCOADHESION IN 0.1N HCl

Formula code	Mean Percentage of microspheres adhering to tissue *(n=3)*					
	0.1N Hcl					
	0.5hr	1hr	2hrs	3hrs	4hrs	5hrs
F_1	69.33 (3.33)	66.67 (3.46)	62.67 (3.69)	60.00 (0.00)	58.67 (3.94)	54.67 (4.03)
F_2	77.33 (2.99)	73.33 (3.15)	69.33 (3.15)	65.33 (5.53)	62.67 (7.33)	57.33 (4.33)
F_3	76.00 (9.12)	70.87 (6.54)	66.67(6.93)	64.00 (6.25)	60.00 (0.00)	54.67 (4.22)
F_4	74.67 (3.09)	70.67 (3.27)	68.00 (0.00)	61.33 (3.77)	62.67 (3.69)	48.00 (8.33)
F_5	89.73 (2.59)	8067 (5.33)	84.00 (4.76)	78.67 (5.87)	78.67 (2.94)	73.33 (3.15)
F_6	84.00 (4.76)	86.67 (5.68)	77.33 (5.97)	78.67 (2.99)	74.67 (3.09)	73.33 (3.15)
F_7	85.33 (2.71)	84.00 (4.75)	80.00 (5.00)	78.67 (2.94)	74.67 (3.09)	68.00 (5.85)
F_8	86.67 (2.59)	84.00 (0.00)	81.33 (2.84)	78.67 (2.94)	74.67 (3.09)	72.00 (5.56)

Table. 25

DATA FOR *IN VITRO* WASH-OFF TEST FOR MUCOADHESION IN PHOSPHATE BUFFER pH 6.8

Formula code	Mean Percentage of microspheres adhering to tissue *(n=3)*			
	Phosphate buffer pH 6.8			
	0.5hr	1hr	2hrs	3hrs
F_1	60.00 (0.00)	54.67 (11.18)	48.00 (8.33)	45.33 (5.09)
F_2	65.33 (3.53)	58.67 (3.94)	52.00 (7.69)	46.67 (4.95)
F_3	64.00 (6.25)	60.00 (0.00)	54.67 (3.69)	49.33 (4.68)
F_4	62.67 3.69)	60.00 (13.33)	50.67 (12.06)	48.00 (8.33)
F_5	78.67 (5.87)	70.67 (18.29)	61.33 (12.06)	57.33 (14.77)
F_6	77.33(2.99)	74.67 (3.69)	61.33 (13.59)	61.33 (3.79)
F_7	80.00 (5.00)	70.67 (2.99)	65.33 (12.74)	58.67 (10.41)

F_8	76.00 (5.26)	73.33 (3.15)	62.67 (13.29)	56.00 (0.00)

Numbers in parenthesis indicates the coefficient of variance (CV) (or) percentage relative standard deviation (%RSD).

CV = (Standard Deviation / Mean)*100

Table. 26

IN VITRO DRUG RELEASE PROFILE FOR FORMULATION F1

Time in hrs	**Cumulative percentage drug release**			**Meam cumulative % drug release ±SD**
	Trial 1	**Trial 2**	**Trial 3**	
0	**0.00**	**0.00**	**0.00**	**0±0**
1	**9.16**	**9.73**	**10.30**	**9.73±0.57**
2	**16.05**	**16.62**	**17.19**	**16.62±0.57**
3	**29.22**	**30.35**	**31.49**	**30.35±1.135**
4	**37.38**	**38.51**	**39.65**	**38.51±1.135**
5	**43.31**	**44.44**	**45.58**	**44.44±1.135**
6	**50.09**	**51.23**	**52.37**	**51.23±1.14**
7	**54.65**	**55.79**	**56.93**	**55.79±1.14**
8	**57.82**	**58.96**	**60.10**	**58.96±1.14**
9	**59.88**	**61.02**	**62.16**	**61.02±1.14**
10	**61.37**	**62.52**	**63.66**	**62.51±1.145**
11	**63.44**	**64.58**	**65.72**	**64.58±1.14**
12	**65.78**	**66.93**	**68.07**	**66.92±1.145**
13	**67.29**	**68.44**	**69.59**	**68.44±1.15**
14	**69.37**	**70.51**	**71.66**	**70.51±1.145**
15	**70.04**	**71.19**	**72.34**	**71.19±1.15**
16	**71.00**	**72.15**	**73.30**	**72.15±1.15**
17	**72.52**	**73.67**	**74.83**	**73.67±1.155**
18	**74.05**	**75.20**	**76.35**	**75.2±1.15**
19	**76.13**	**77.29**	**78.44**	**77.28±1.155**
20	**79.63**	**80.78**	**81.94**	**80.78±1.155**
21	**80.61**	**81.76**	**82.92**	**81.76±1.155**
22	**81.59**	**82.74**	**83.90**	**82.74±1.155**
23	**84.25**	**85.41**	**86.57**	**85.41±1.16**
24	**86.64**	**87.80**	**88.96**	**87.8±1.16**

Figure.8

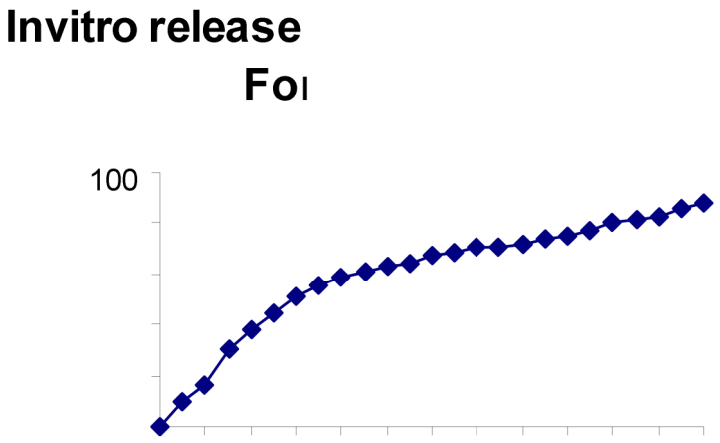


Figure .9

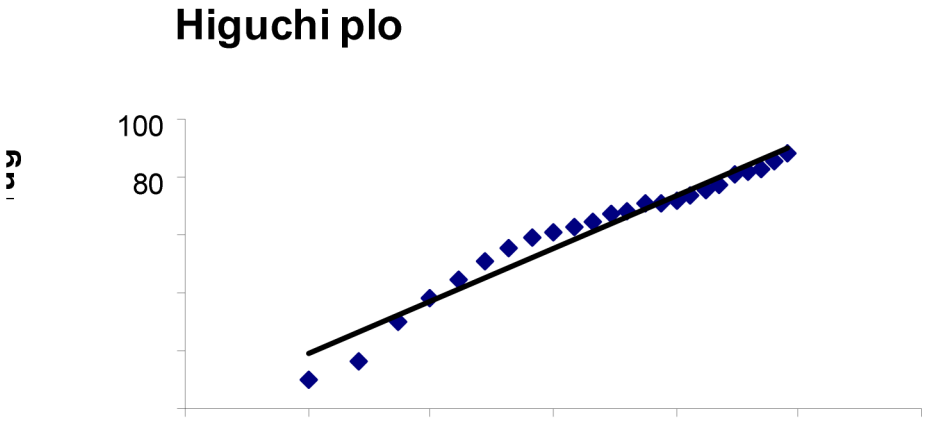


Figure .10

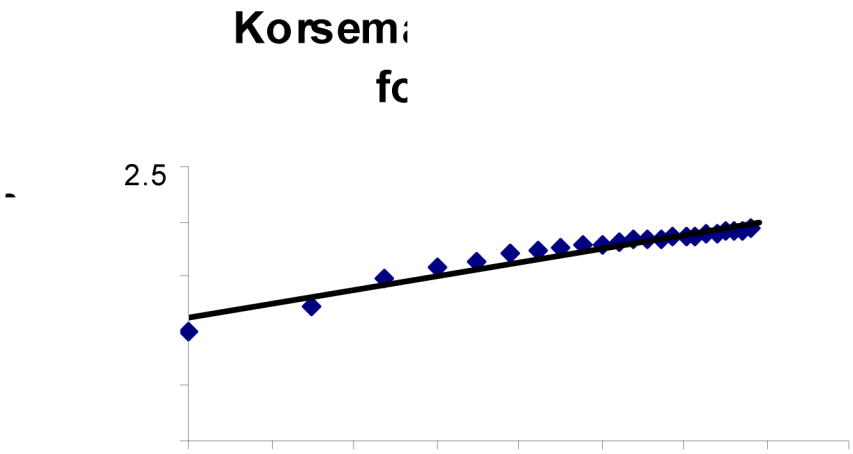


Figure .11

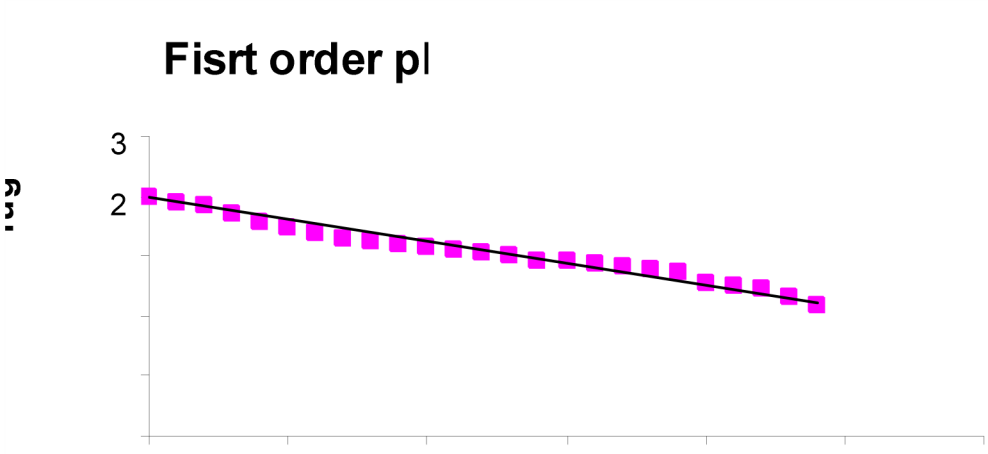


Table 27

IN VITRO DRUG RELEASE PROFILE FOR FORMULATION F2

Time in hrs	Cumulative percentage drug release			Meam cumulative % drug release ±SD
	Trial 1	Trial 2	Trial 3	
0	0.00	0.00	0.00	0±0
1	7.44	8.01	8.59	8.013±0.575
2	14.04	14.61	15.19	14.61±0.575
3	25.53	26.66	27.80	26.66±1.135
4	35.36	36.50	37.64	36.5±1.14
5	40.74	41.87	43.01	41.87±1.135
6	45.84	46.98	48.12	46.98±1.14
7	49.55	50.69	51.83	50.69±1.14
8	51.59	52.74	53.88	52.73±1.145
9	54.20	55.34	56.48	55.34±1.14
10	58.21	59.36	60.50	59.35±1.145
11	59.71	60.85	62.00	60.85±1.145
12	60.93	62.08	63.22	62.07±1.145
13	62.72	63.86	65.01	63.86±1.145
14	64.22	65.37	66.52	65.37±1.15
15	66.30	67.44	68.59	67.44±1.145
16	68.37	69.52	70.67	69.52±1.15
17	70.17	71.32	72.47	71.32±1.15
18	72.26	73.41	74.56	73.41±1.15
19	74.06	75.22	76.37	75.21±1.155
20	75.88	77.03	78.19	77.03±1.155
21	77.13	78.29	79.44	78.28±1.155
22	78.67	79.83	80.98	79.82±1.155
23	82.45	83.61	84.77	83.61±1.16
24	84.56	85.72	86.88	85.72±1.16

Figure.12

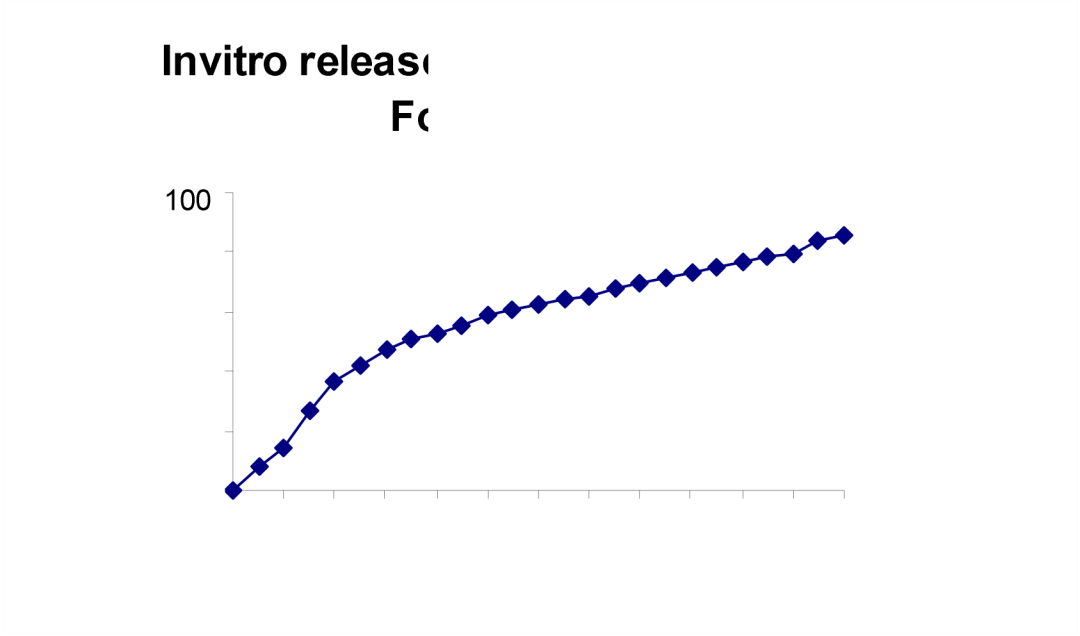


Figure .13

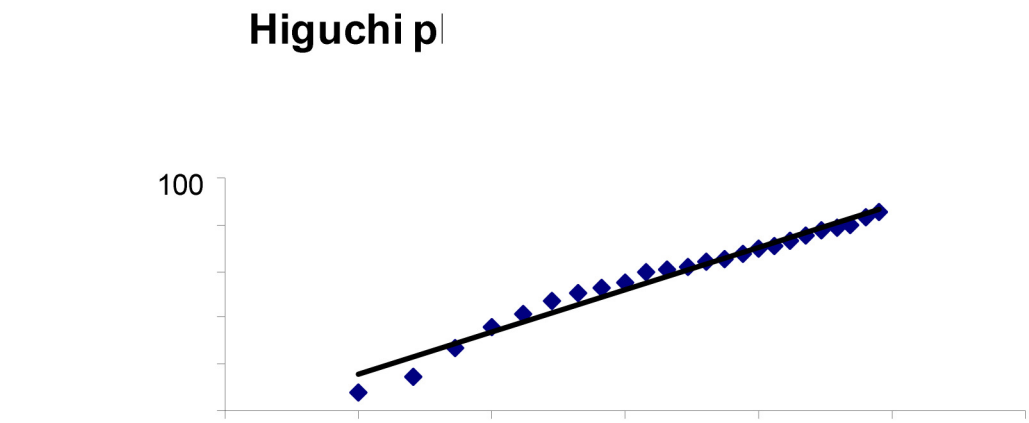


Figure. 14

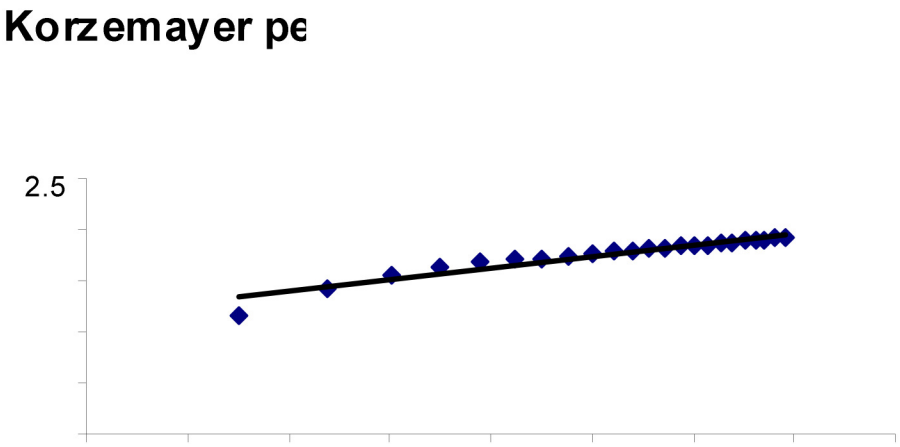


Figure.15

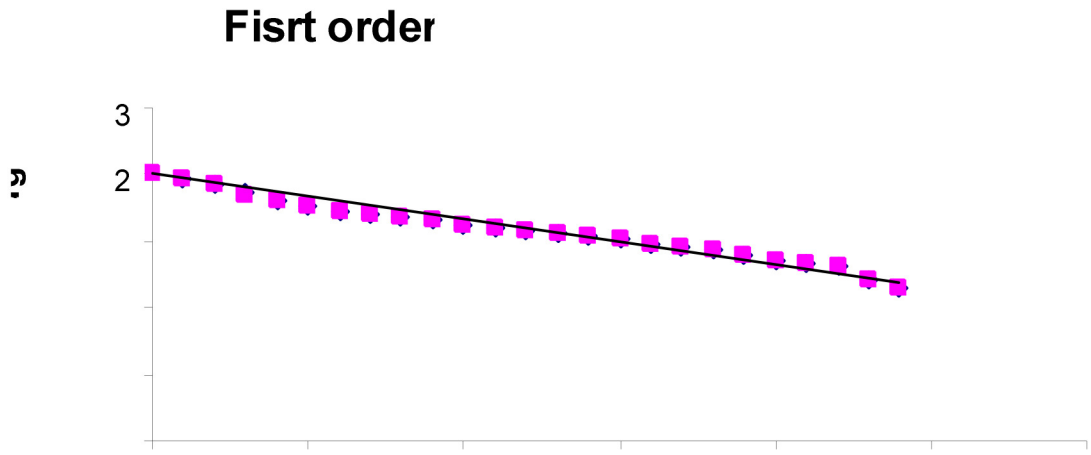


Table. 28

INVITRO DRUG RELEASE PROFILE FOR FORMULATION F_3

Time in hrs	Cumulative percentage drug release			Meam cumulative % drug release ±SD
	Trial 1	Trial 2	Trial 3	
0	0.00	0.00	0.00	0±0
1	8.87	9.44	10.02	9.44±0.575
2	16.05	16.62	17.19	16.62±0.575
3	29.22	30.35	31.49	30.35±1.135
4	37.37	38.51	39.65	38.51±1.14
5	40.22	41.36	42.50	41.36±1.14
6	43.36	44.50	45.64	44.5±1.14
7	46.78	47.92	49.06	47.92±1.14
8	51.62	52.76	53.90	52.76±1.14
9	54.78	55.92	57.06	55.92±1.14
10	57.39	58.53	59.67	58.53±1.14
11	59.72	60.86	62.01	60.86±1.145
12	62.06	63.20	64.35	63.20±1.145
13	65.80	66.95	68.09	66.94±1.145
14	67.59	68.74	69.89	68.74±1.15
15	69.39	70.54	71.68	70.53±1.145
16	70.90	72.05	73.20	72.05±1.15
17	72.43	73.58	74.73	73.58±1.15
18	73.67	74.82	75.97	74.82±1.15
19	75.48	76.63	77.78	76.63±1.15
20	77.01	78.16	79.32	78.16±1.155
21	77.98	79.14	80.29	79.13±1.155
22	78.95	80.11	81.27	80.11±1.16
23	80.77	81.93	83.09	81.93±1.16
24	82.31	83.47	84.63	83.47±1.16

Figure .16

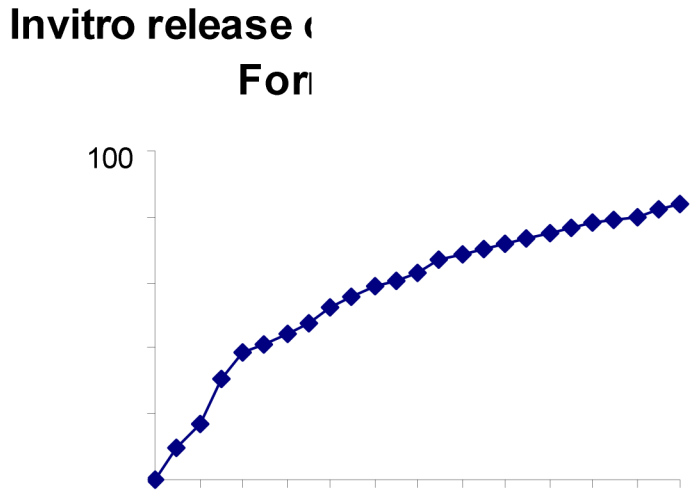


Figure .17

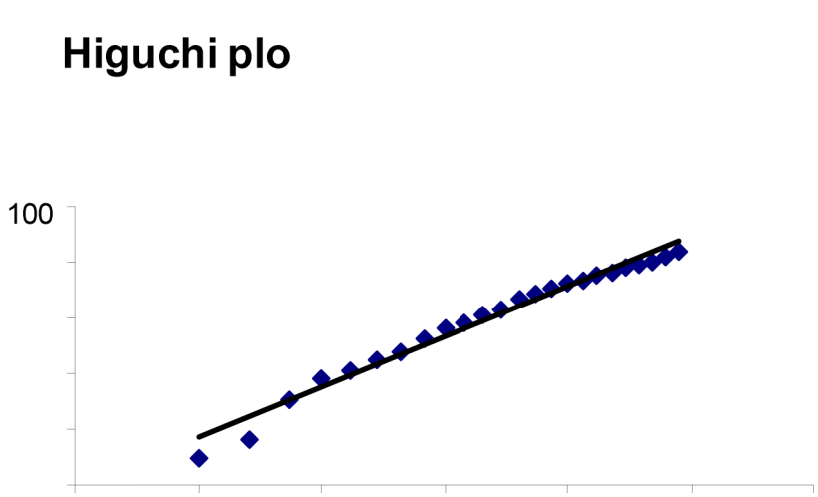


Figure .18

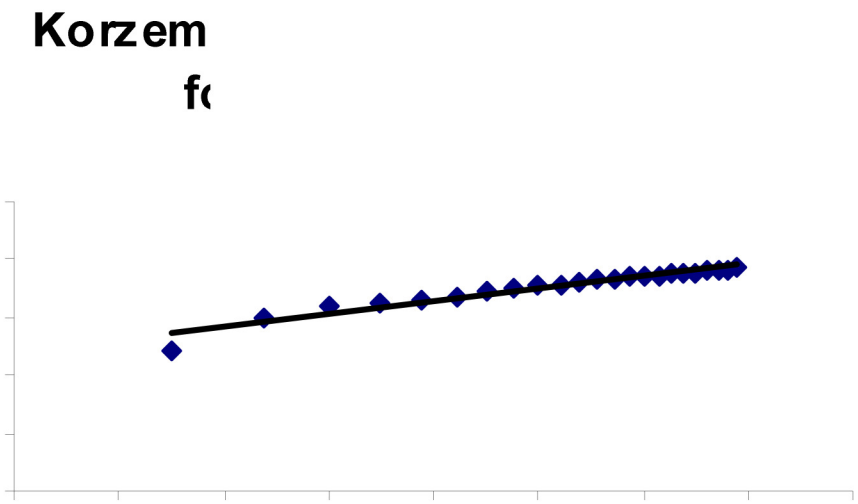


Figure .19

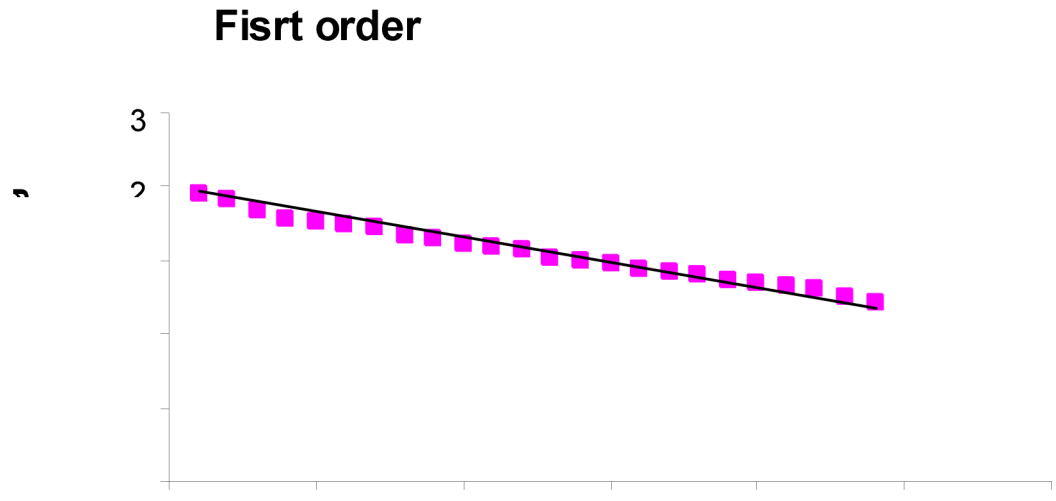


Table. 29

IN VITRO DRUG RELEASE PROFILE FOR FORMULATION F_4

Time in hrs	Cumulative percentage drug release			Meam cumulative % drug release ±SD
	Trial 1	Trial 2	Trial 3	
0	0.00	0.00	0.00	0±0
1	8.30	8.87	9.44	08.87±0.57
2	11.18	11.75	12.33	11.75±0.575
3	25.19	26.33	27.46	26.32±1.135
4	29.71	30.84	31.98	30.84±1.135
5	33.67	34.81	35.95	34.81±1.14
6	38.77	39.90	41.04	39.90±1.135
7	42.75	43.89	45.03	43.89±1.14
8	47.59	48.73	49.87	48.73±1.14
9	51.87	53.01	54.15	53.01±1.14
10	55.32	56.47	57.61	56.46±1.145
11	56.82	57.97	59.11	57.96±1.145
12	58.04	59.19	60.33	59.18±1.145
13	58.99	60.13	61.28	60.13±1.145
14	60.49	61.64	62.79	61.64±1.15
15	62.28	63.43	64.58	63.43±1.15
16	64.64	65.79	66.94	65.79±1.15
17	66.43	67.59	68.74	67.58±1.155
18	68.80	69.95	71.10	69.95±1.15
19	70.88	72.04	73.19	72.03±1.155
20	72.69	73.85	75.00	73.84±1.155
21	73.95	75.10	76.26	75.10±1.155
22	74.92	76.08	77.24	76.08±1.16
23	75.90	77.06	78.22	77.06±1.16
24	81.65	82.81	83.97	82.81±1.16

Figure .20

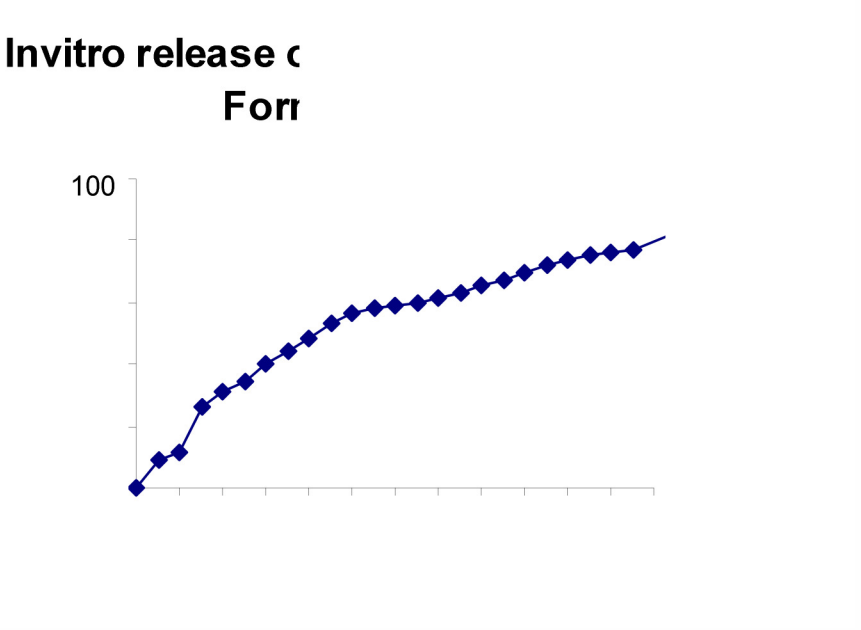


Figure. 21

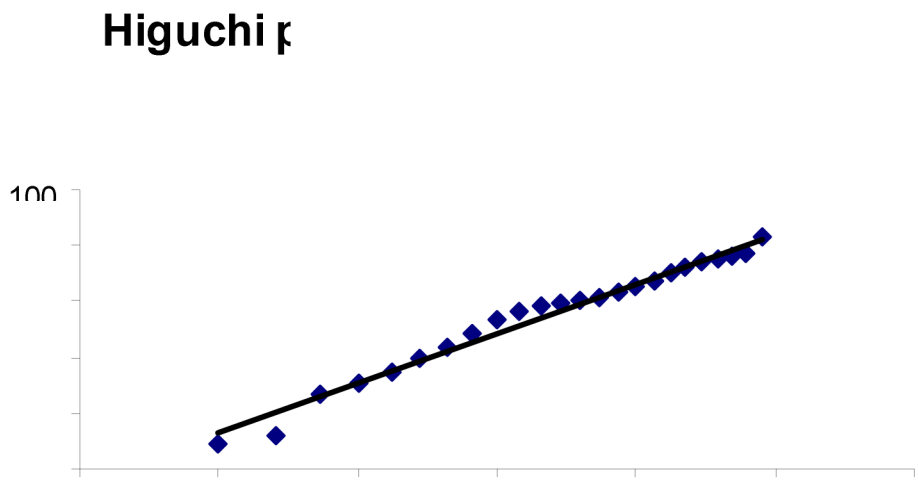


Figure. 22

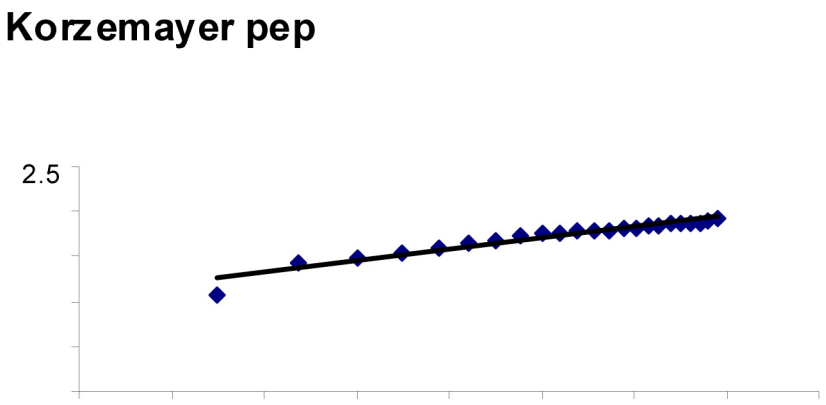


Figure. 23

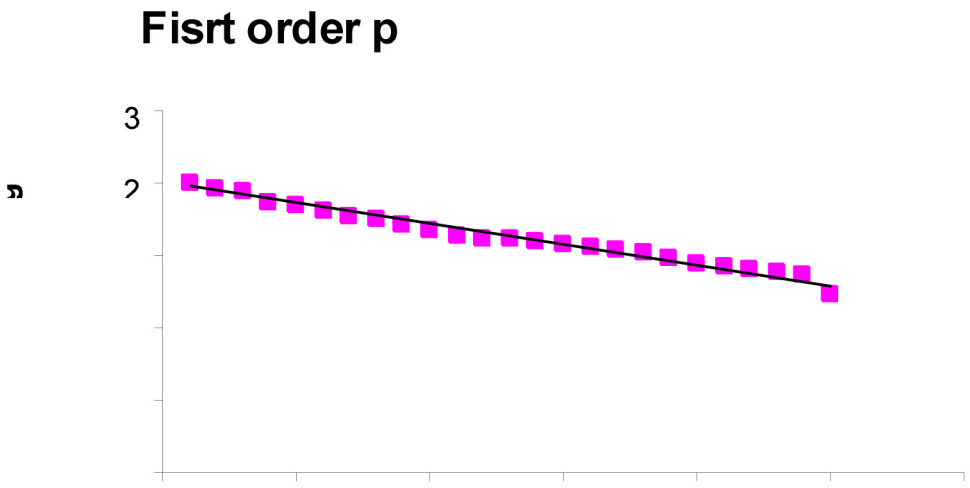


Table. 30

IN VITRO DRUG RELEASE PROFILE FOR FORMULATION F_5

Time in hrs	Cumulative percentage drug release			Meam cumulative % drug release ±SD
	Trial 1	Trial 2	Trial 3	
0	0.00	0.00	0.00	0±0
1	8.87	9.44	10.02	9.443±0.575
2	12.04	12.61	13.19	12.61±0.575
3	27.73	28.87	30.00	28.86±1.135
4	33.09	34.23	35.36	34.22±1.135
5	37.06	38.20	39.34	38.2±1.14
6	41.88	43.02	44.16	43.02±1.14
7	46.15	47.29	48.43	47.29±1.14
8	49.87	51.01	52.15	51.01±1.14
9	53.04	54.18	55.32	54.18±1.14
10	54.53	55.67	56.82	55.67±1.145
11	56.59	57.73	58.87	57.73±1.14
12	58.93	60.07	61.22	60.07±1.145
13	60.43	61.58	62.72	61.57±1.145
14	62.50	63.65	64.79	63.64±1.145
15	63.17	64.32	65.47	64.32±1.15
16	64.12	65.27	66.42	65.27±1.15
17	65.64	66.79	67.94	66.79±1.15
18	69.96	71.11	72.26	71.11±1.15
19	72.04	73.20	74.35	73.19±1.155
20	73.86	75.01	76.17	75.01±1.155
21	76.51	77.67	78.82	77.66±1.155
22	78.33	79.49	80.65	79.49±1.16
23	79.32	80.48	81.63	80.47±1.155
24	81.42	82.58	83.74	82.58±1.16

Figure. 24

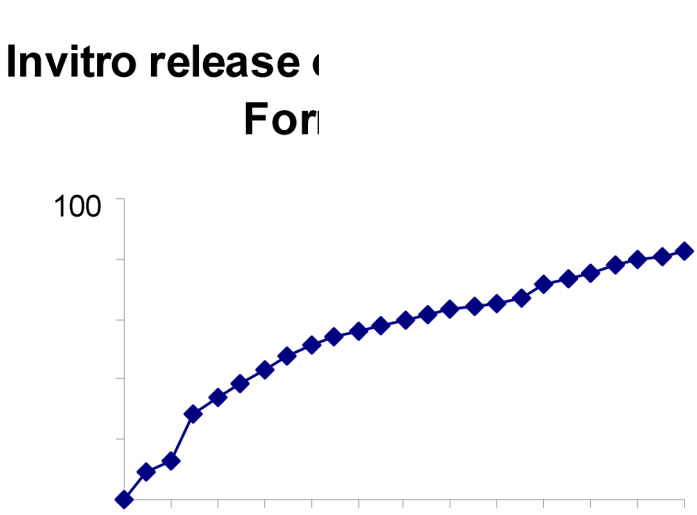


Figure. 25

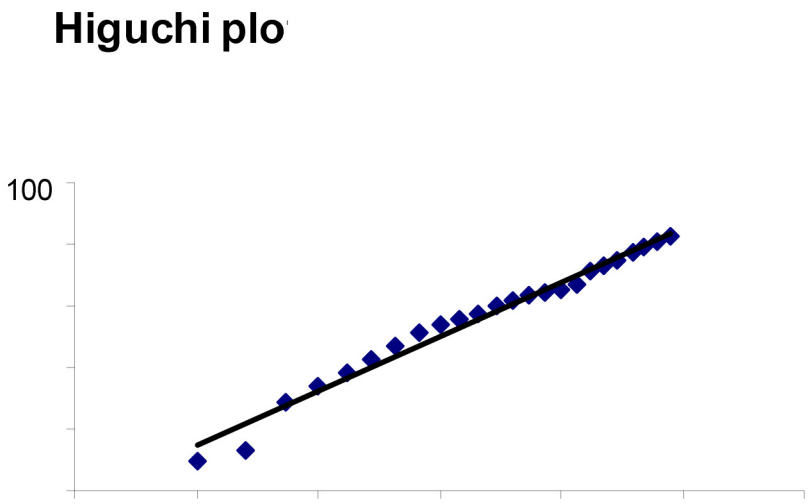


Figure. 26

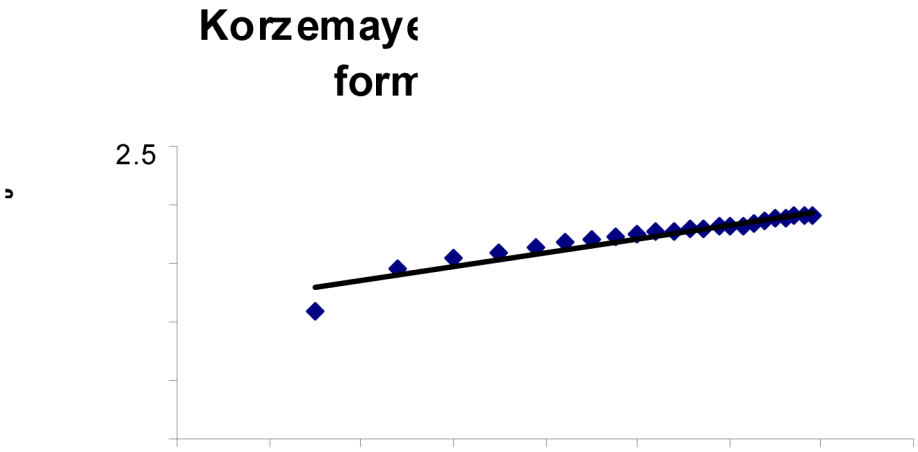


Figure. 27

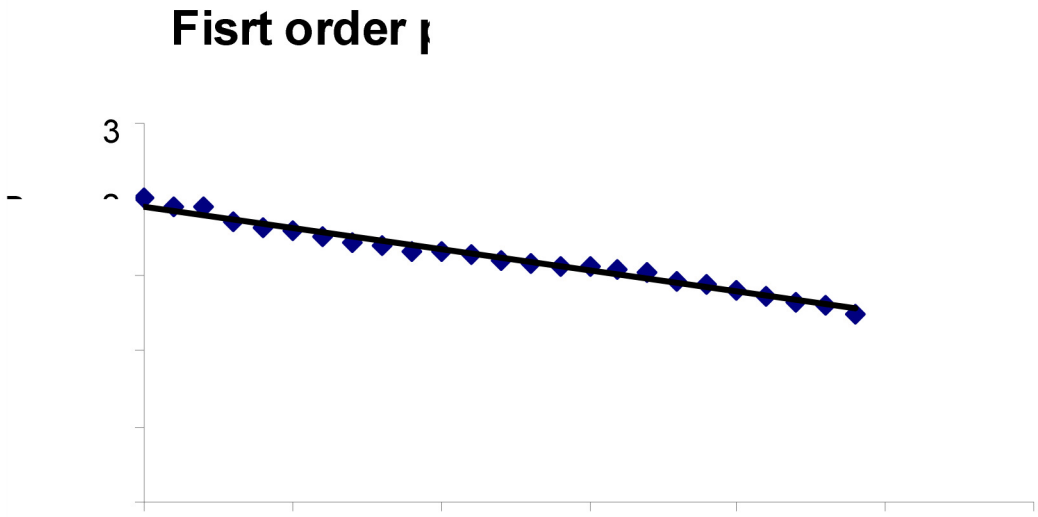


Table. 31

IN VITRO DRUG RELEASE PROFILE FOR FORMULATION F6

Time in hrs	Cumulative percentage drug release			Mean cumulative % drug release ±SD
	Trial 1	Trial 2	Trial 3	
0	0.00	0.00	0.00	0±0
1	8.87	9.44	10.02	9.443±0.575
2	11.75	12.33	12.90	12.32±0.575
3	25.49	26.62	27.75	26.62±1.13
4	27.48	28.61	29.75	28.61±1.135
5	29.19	30.33	31.47	30.33±1.14
6	34.56	35.70	36.83	35.69±1.135
7	41.90	43.03	44.17	43.03±1.135
8	45.04	46.19	47.33	46.18±1.145
9	49.88	51.02	52.17	51.02±1.145
10	51.93	53.07	54.21	53.07±1.14
11	53.70	54.84	55.99	54.84±1.145
12	55.75	56.90	58.04	56.89±1.145
13	57.25	58.40	59.54	58.39±1.145
14	58.47	59.62	60.77	59.62±1.15
15	59.69	60.84	61.99	60.84±1.15
16	61.48	62.63	63.78	62.63±1.15
17	63.55	64.70	65.85	64.7±1.15
18	67.00	68.15	69.30	68.15±1.15
19	68.55	69.70	70.86	69.70±1.155
20	70.91	72.07	73.22	72.06±1.155
21	72.16	73.32	74.48	73.32±1.16
22	73.98	75.13	76.29	75.13±1.155
23	77.75	78.91	80.07	78.91±1.16
24	79.58	80.74	81.90	80.74±1.16

Figure. 28

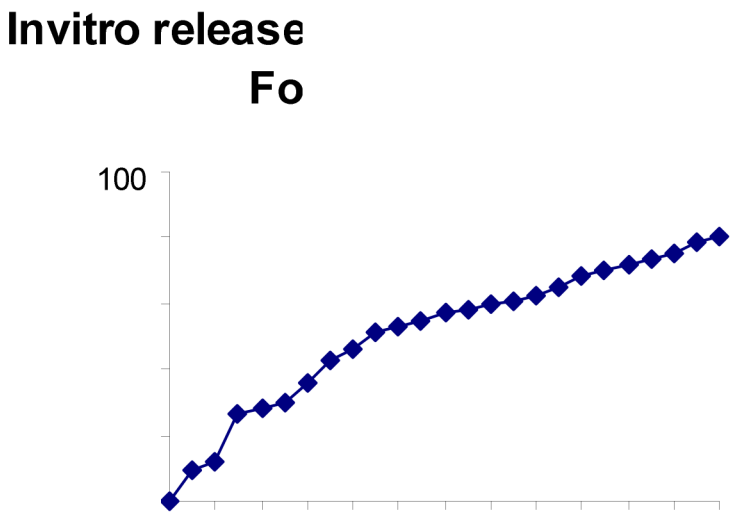


Figure 29

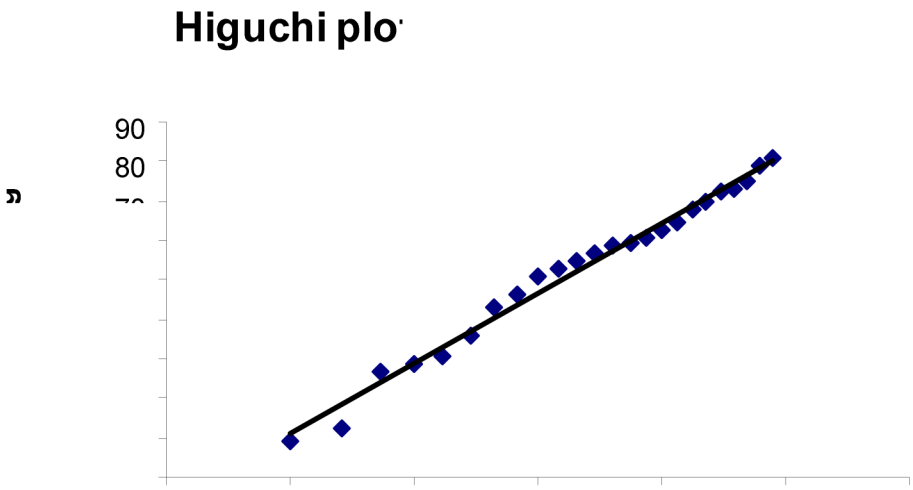


Figure. 30

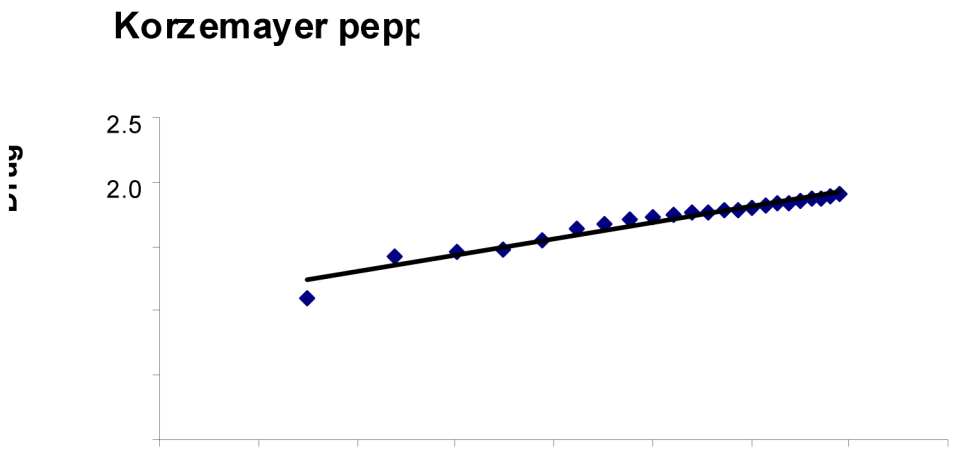


Figure. 31

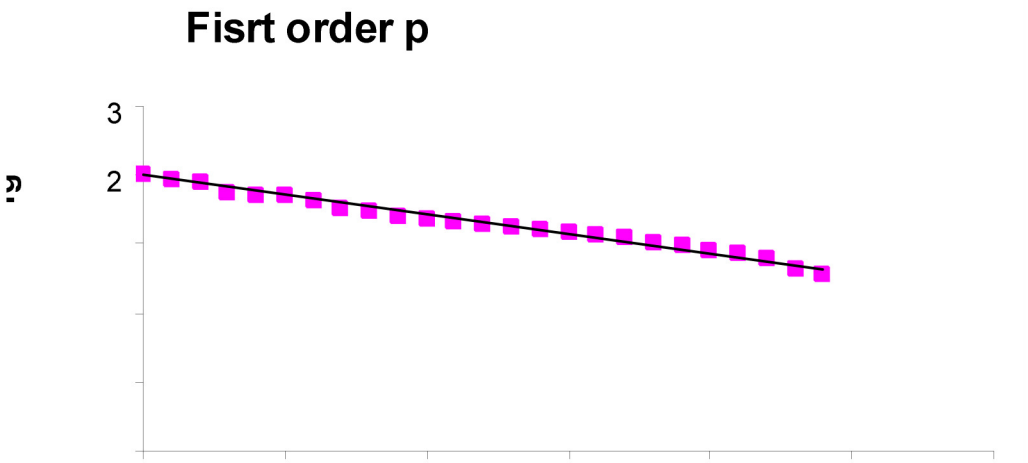


Table. 32

IN VITRO DRUG RELEASE PROFILE FOR FORMULATION F7

Time in hrs	Cumulative percentage drug release			Meam cumulative % drug release ±SD
	Trial 1	Trial 2	Trial 3	
0	0.00	0.00	0.00	0±0
1	10.02	10.59	11.16	10.59±0.57
2	14.04	14.62	15.19	14.61±0.575
3	30.30	31.43	32.57	31.43±1.135
4	35.38	36.52	37.65	36.51±1.135
5	38.51	39.65	40.78	39.64±1.135
6	42.49	43.63	44.76	43.62±1.135
7	48.16	49.30	50.44	49.3±1.14
8	50.19	51.33	52.48	51.33±1.145
9	52.24	53.38	54.52	53.38±1.14
10	53.72	54.86	56.01	54.86±1.145
11	55.77	56.91	58.06	56.91±1.145
12	57.82	58.97	60.11	58.96±1.145
13	59.32	60.47	61.61	60.46±1.145
14	61.66	62.81	63.96	62.81±1.15
15	65.13	66.28	67.43	66.28±1.15
16	66.08	67.23	68.38	67.23±1.15
17	67.88	69.03	70.18	69.03±1.15
18	70.52	71.67	72.82	71.67±1.15
19	72.04	73.19	74.35	73.19±1.155
20	74.13	75.28	76.44	75.28±1.155
21	75.10	76.26	77.41	76.25±1.155
22	76.63	77.79	78.95	77.79±1.16
23	79.29	80.45	81.61	80.45±1.16
24	81.67	82.83	83.99	82.83±1.16

Figure.32

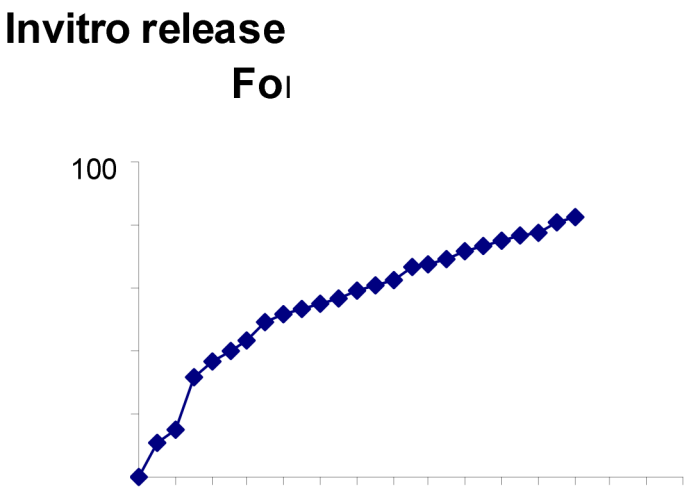


Figure. 33

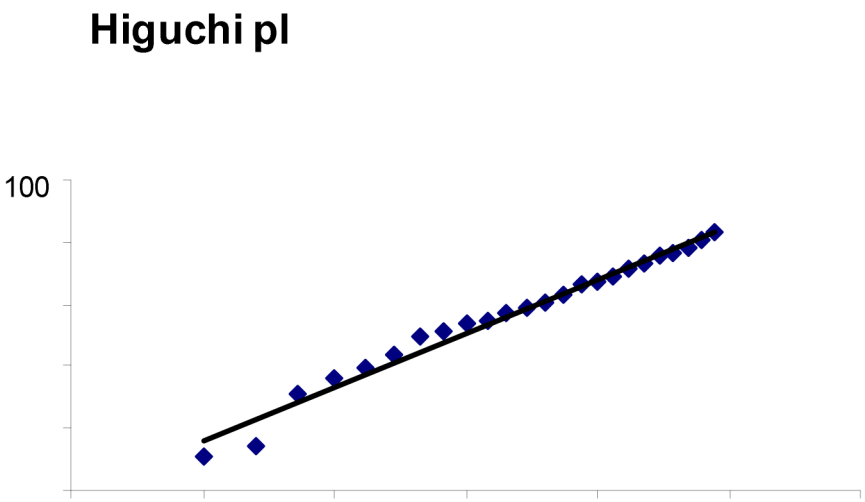


Figure. 34

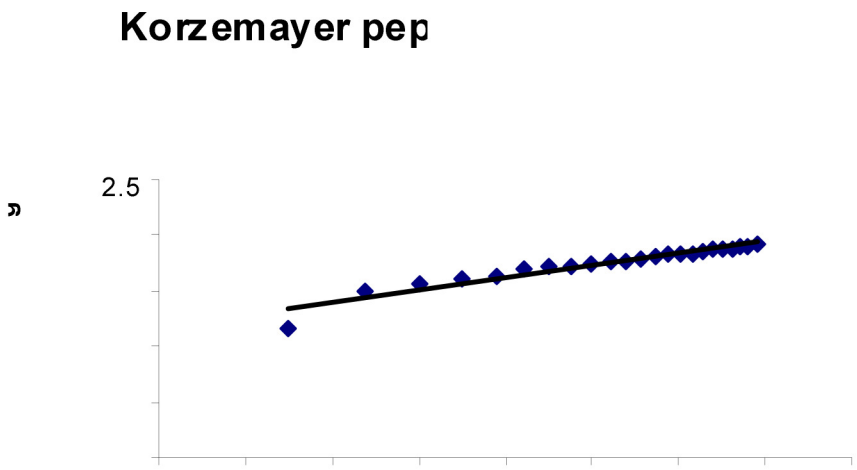


Figure. 35

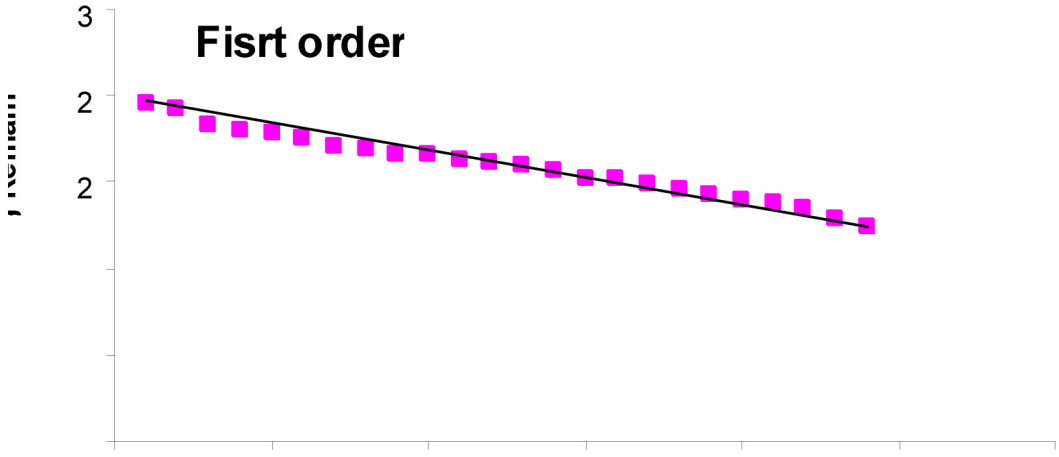


Table. 33

IN VITRO DRUG RELEASE PROFILE FOR FORMULATION F8

Time in hrs	Cumulative percentage drug release			Meam cumulative % drug release ±SD
	Trial 1	Trial 2	Trial 3	
0	0.00	0	0.00	0±0
1	9.16	9.73	10.30	9.73±0.57
2	14.04	14.62	15.19	14.61±0.575
3	25.53	26.67	27.80	26.66±1.135
4	29.76	31.71	32.03	31.16±1.228
5	32.32	33.46	34.59	33.45±1.135
6	34.32	35.46	36.60	35.46±1.14
7	38.57	39.71	40.85	39.71±1.14
8	43.11	44.25	45.39	44.25±1.14
9	46.82	47.96	49.10	47.96±1.14
10	49.69	50.84	51.98	50.83±1.145
11	52.01	53.16	54.30	53.15±1.145
12	54.06	55.2	56.35	55.20±1.145
13	55.83	56.97	58.12	56.97±1.145
14	60.40	61.55	62.70	61.55±1.15
15	62.19	63.34	64.48	63.33±1.145
16	63.69	64.84	65.99	64.84±1.15
17	64.92	66.07	67.23	66.07±1.155
18	66.71	67.87	69.02	67.86±1.155
19	68.51	69.67	70.82	69.66±1.155
20	70.87	72.03	73.18	72.02±1.155
21	72.68	73.83	74.99	73.83±1.155
22	75.05	76.2	77.36	76.20±1.155
23	77.42	78.58	79.74	78.58±1.16
24	79.24	80.4	81.56	80.4±1.16

Figure. 36

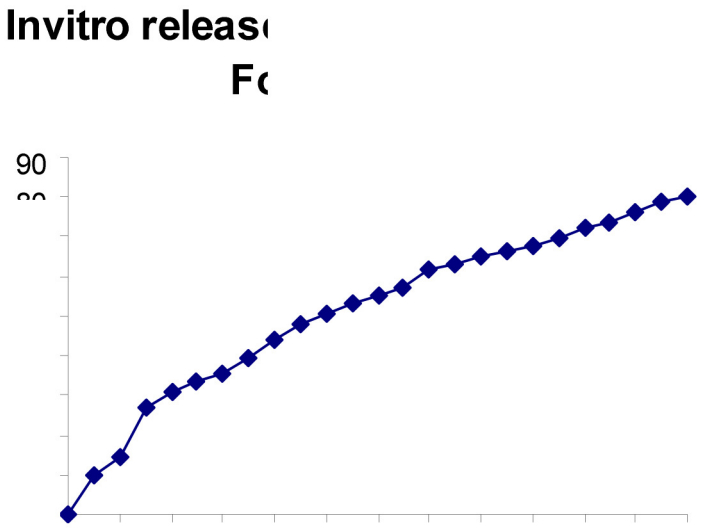


Figure. 37

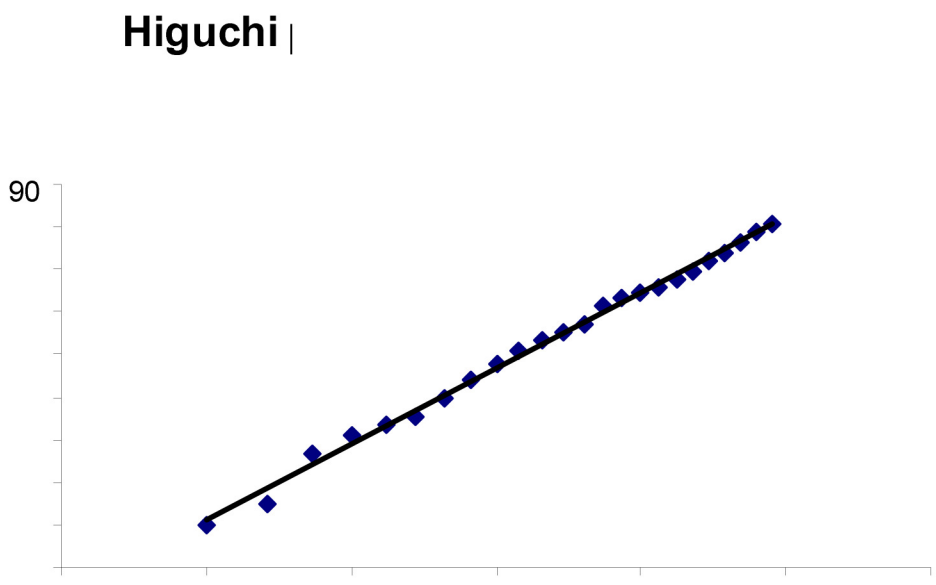


Figure. 38

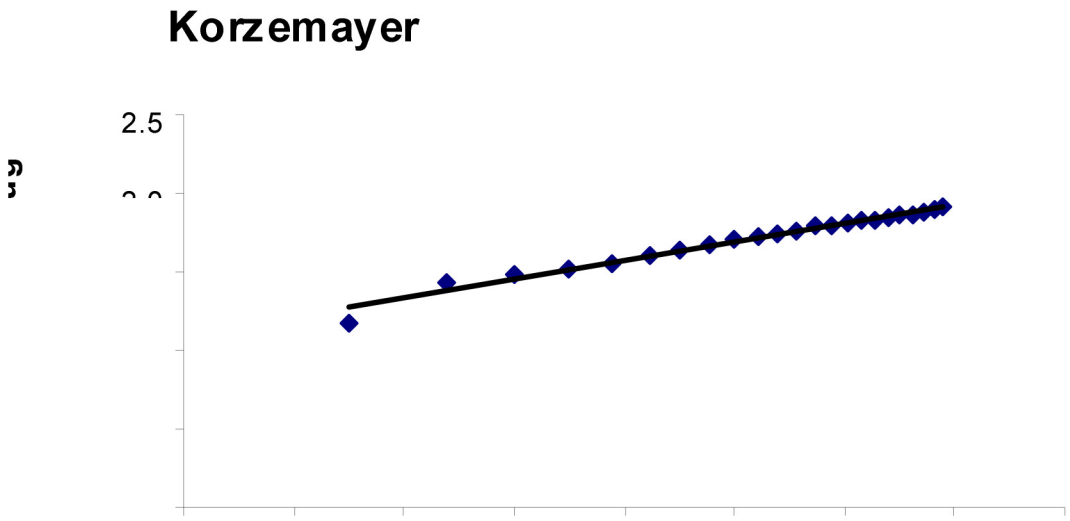


Figure. 39

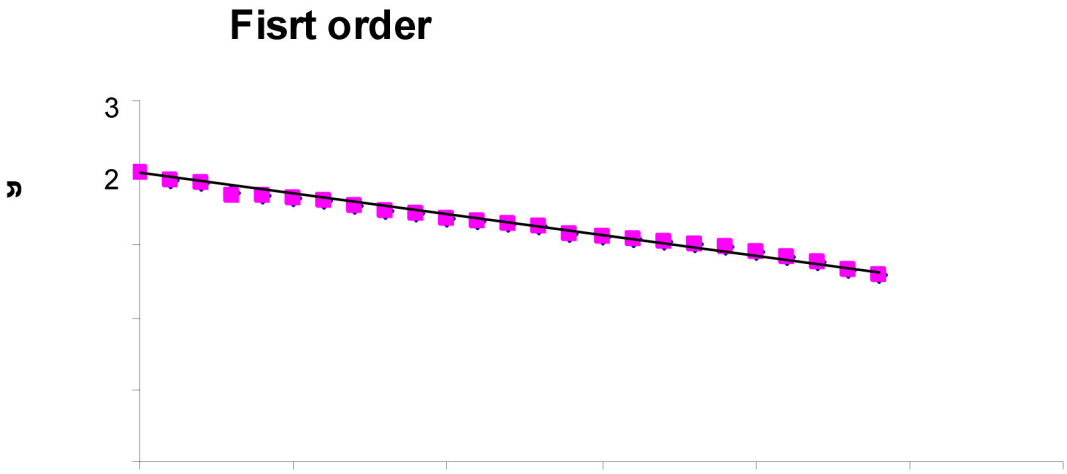


Table 34

IN VITRO RELEASE KINETIC DATA FOR VENLAFAXINE HCL MUCOADHESIVE MICROSPHERES

Formula code	$t_{80\%}$	Zero order		First order		Higuchi's	Korsmeyer-Peppas	
		K_0	r	K_1	r	r	n	R
F_1	19.8	3.0111	0.92517	-0.0328	-0.9867	0.9833	0.6153	0.9627
F_2	22.05	3.0114	0.9413	-0.0307	-0.9899	0.9888	0.6593	0.9656
F_3	21.23	2.9960	0.9413	-0.3054	-0.9944	0.9910	0.6193	0.9726
F_4	23.27	2.9550	0.9521	-0.0275	-0.9909	0.9913	0.6692	0.9751
F_5	20.86	2.9422	0.9491	-0.0285	-0.9913	0.9910	0.6408	0.9698
F_6	22.82	2.9382	0.9520	-0.0267	-0.9927	0.9933	0.6576	0.9828
F_7	21.75	2.8704	0.9474	-0.0280	-0.9913	0.9919	0.5972	0.9714
F_8	23.88	2.9423	0.9503	-0.0270	-0.9967	0.9969	0.6333	0.9901

K_0 – Zero order rate constant

K_1 – First order rate constant

r – Coefficient of Correlation

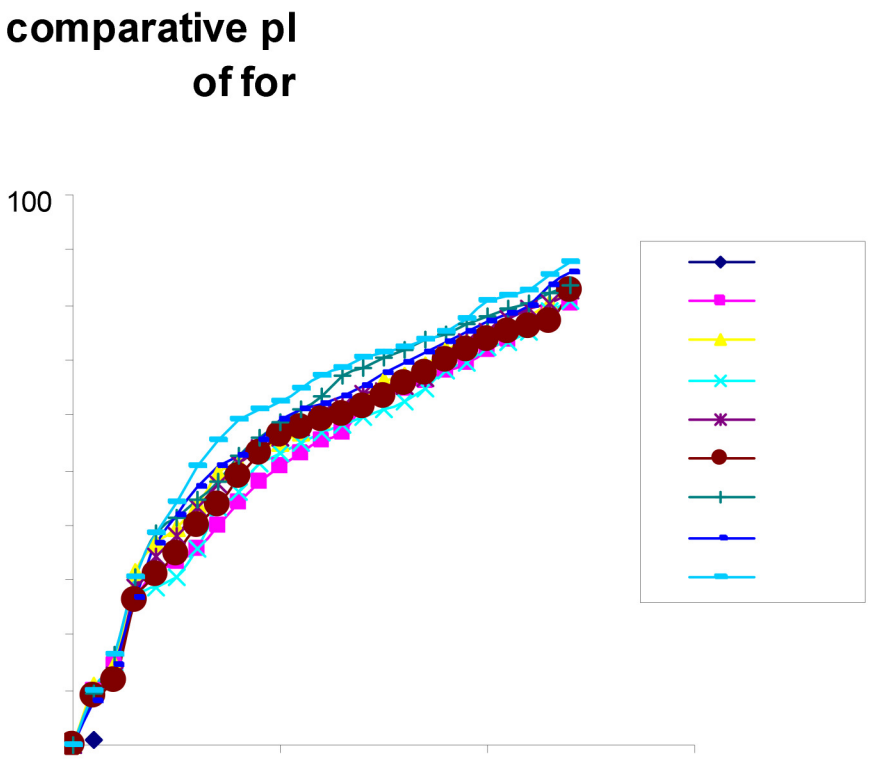
n- Diffusion exponent

Table. 35

Time in hrs	Mean Cumulative % drug release of F1	Mean Cumulative % drug release of F2	Mean Cumulative % drug release of F3	Mean Cumulative % drug release of F4	Mean Cumulative % drug release of F5	Mean Cumulative % drug release of F6	Mean Cumulative % drug release of F7	Mean Cumulative % drug release of F8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	9.73	8.01	9.44	8.87	9.44	9.44	10.59	9.73
2	16.62	14.61	16.62	11.75	12.61	12.33	14.62	14.62
3	30.35	26.66	30.35	26.33	28.87	26.62	31.43	26.67
4	38.51	38.07	39.77	31.04	35.59	29.39	37.50	31.71
5	44.44	41.87	41.36	34.81	38.20	30.33	39.65	33.46
6	51.23	46.98	44.50	39.90	43.02	35.70	43.63	35.46
7	55.79	50.69	47.92	43.89	47.29	43.03	49.30	39.71
8	59.96	52.74	52.76	48.73	51.01	46.19	51.33	44.25
9	61.02	55.34	55.92	53.01	54.18	51.02	53.38	47.96
10	62.52	59.36	58.53	56.47	55.67	53.07	54.86	50.84
11	64.58	60.85	60.86	57.97	57.73	54.84	56.91	53.16
12	66.93	63.08	63.20	59.19	60.07	56.90	58.97	55.20
13	68.44	63.86	66.95	60.13	61.58	58.40	60.47	56.97
14	70.51	65.37	68.74	61.64	63.65	59.62	62.81	61.55
15	71.19	67.44	70.54	63.43	64.32	60.84	66.28	63.34
16	72.60	70.20	72.05	65.79	65.27	62.63	67.23	64.84
17	73.67	71.32	73.58	67.59	66.79	64.70	69.03	66.07
18	75.20	73.41	74.82	69.95	71.11	68.15	71.67	67.87
19	77.29	75.22	76.63	72.04	73.20	69.70	73.19	69.67
20	80.78	77.03	78.16	73.85	75.01	72.07	75.28	72.03
21	81.76	78.29	79.14	75.10	77.67	73.32	76.26	73.83
22	82.74	79.83	80.11	76.08	79.49	75.13	77.79	76.20
23	85.41	83.61	81.93	77.06	80.48	78.91	80.45	78.58
24	87.80	85.72	83.47	82.81	82.58	80.74	82.83	80.40

COMPARATIVE CUMULATIVVE % DRUG RELEASE PROFILE OF F1-F8

Figure. 40



SCANNING ELECTRON MICROGRAPH (SEM) OF THE PREPARED MUCOADHESIVE MICROSPHERES OF VENLAFAXINE HCL FORMULATION

Figure. 41

Figure. 42

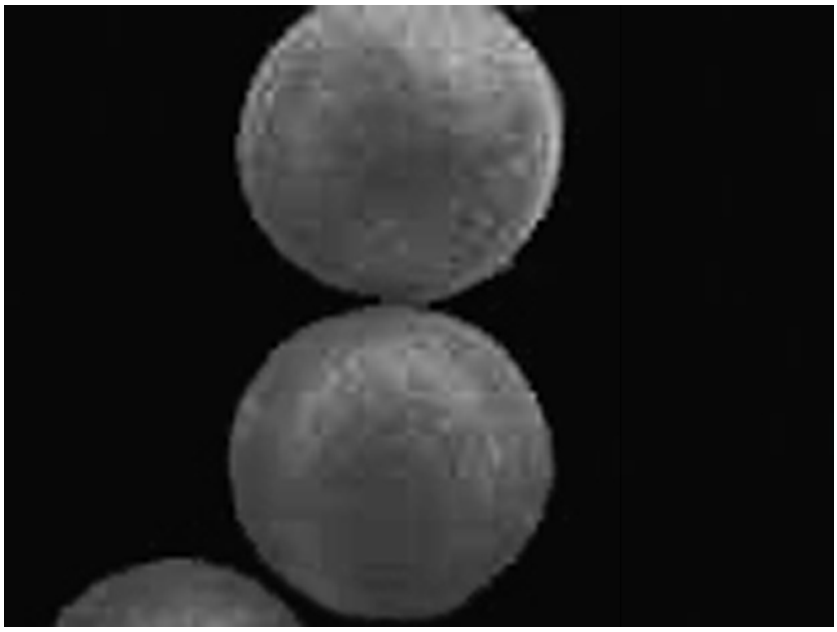


Figure. 43

SURFACE VIEW OF THE PREPARED MUCOADHESIVE MICROSPHERES

8. 1 Statistical Optimization technique

The optimization phase was designed statistically using 2^3 factorial design in which three variables namely concentrations of polymers such as EC, Eudragit RS100 and HPMC K4M were kept at two levels. Except the optimization phase whose purpose was validated by extra design check point and main interactive influences were tested using statistical methods. The eight formulations of optimization phase were categorized in to four groups for ease of analysis and comparison as follows:

1. Group I : All variables at low level (Formulation F_1).
2. Group II : Any one of three variables at high level (Formulations F_2, F_3, F_5).
3. Group III : Any two of three variables at high level (Formulations F_4, F_6, F_7).
4. Group IV : All three variables at high level (Formulation F_8).

Although all formulation were analyzed for uniformity of release pattern, amount of drug release at the end of 24 hours, and mechanism of drug release, and all of these parameters were considered for selection of best formulation in the optimization phase, only release rate and $t_{80\%}$ values were used for comparative analysis as they characterize the entire kinetic profile.

Compare to base line response of Group-I formulation the $t_{80\%}$ values of all formulations in Group-II were relatively high. It indicates that every polymer has the ability to sustain and retard the release at high concentration, though the magnitude of their impact was chiefly indicated by their extent of solubility and swellability. The formulation F_2, F_3 and F_5 contains higher proportion of EC, Eudragit RS100 and HPMC K4M respectively. Based on the $t_{80\%}$ value of the above formulations, the degree of retardency is in the order of EC > HPMC K4M >Eudragit RS100.

The formulation F_4, F_6 and F_7 containing any two of three polymer in higher concentration namely Eudragit RS100 & EC, HPMC K4M & EC and Eudragit RS100& HPMC K4M. The observed $t_{80\%}$ value demonstrated the influence in retarding the release as follows HPMC K4M & EC > Eudragit RS100 & EC > Eudragit RS100 & HPMC K4M. The formulation F_8 containing three polymers in high concentration showed the. $t_{80\%}$ value of 23.88 hours

Table: 34

Formula Code	Venlafaxine HCl (mg)	EC (mg)	Eudragit RS100 (mg)	HPMC K4M (mg)	$T_{80\%}$ (hrs)	Response
F_1	500	750	100	200	19.8	Y_1
F_2	500	1000	100	200	22.05	Y_2
F_3	500	750	200	200	21.23	Y_3
F_4	500	1000	200	200	23.27	Y_4
F_5	500	750	100	300	20.86	Y_5
F_6	500	1000	100	300	22.82	Y_6
F_7	500	750	200	300	21.75	Y_7
F_8	500	1000	200	300	23.88	Y_8

MAIN EFFECTS

Effect of EC $= ((Y_2-Y_1) + (Y_4-Y_3) + (Y_6-Y_5) + (Y_8-Y_7)) / 4$

$= ((22.05-19.8) + (23.27-21.23) + (22.82-20.86) + (23.88-21.75) / 4$

$= +2.095$

Effect of Eudragit RS100 $= ((Y_3-Y_1) + (Y_4-Y_2) + (Y_7-Y_5) + (Y_8-Y_6)) / 4$

$= ((21.23-19.80) + (23.27-22.05) + (21.75-20.86) + (23.88-22.82)) / 4$

$= +1.15$

Effect of HPMC K4M $= ((Y_5-Y_1) + (Y_6-Y_2) + (Y_7-Y_3) + (Y_8-Y_4)) / 4$

$= ((20.86-19.80) + (22.82-22.05) + (21.75-21.23) + (23.88-23.27)) /4$

$= +0.74$

Effect of EC x EUDRAGIT RS100

High level (+) = $((Y_4-Y_3) + (Y_8-Y_7)) / 2$

= $((23.27-21.23) + (23.88-21.75) / 2$

= 2.085

Low level (-) = $((Y_2-Y_1) + (Y_6-Y_5)) / 2$

= $((22.05-19.80) + (22.82-20.86)) / 2$

= 2.105

Effect of HPMC x EC

High level = $((Y_8-Y_4) + (Y_7-Y_3)) / 2$

= $((23.88-23.27) + (21.75-21.23)) / 2$

= 0.565

Low level = $((Y_6-Y_2) + (Y_5-Y_1)) / 2$

= $((22.82-22.05) + (20.86-19.80)) / 2$

= 0.915

Effect of HPMC K4M x EUDRAGIT RS100

High level $= ((Y_6-Y_2) + (Y_8-Y_4)) / 2$

$= ((22.82-22.05) + (23.88-23.27)) / 2$

$= 0.69$

Low level $= ((Y_5-Y_1) + (Y_7-Y_3)) / 2$

$= ((20.86-19.80) + (21.75-21.23)) / 2$

$= 0.79$

Effect of EC x EUDRAGIT RS100x HPMC

High level $= ((Y_8-Y_7) + (Y_6-Y_5)) / 2$

$= ((23.88-21.75) + (22.82-20.86)) / 2$

$= 2.045$

Low level $= ((Y_4-Y_3) + (Y_2-Y_1)) / 2$

$= ((23.27-21.23) + (22.05-19.80)) / 2$

$= 2.145$

EC x HPMC =1/2 difference

= (High level – low level) / 2

= (0.565-0.915) / 2

= -0.175

HPMC x EUDRAGIT RS100 = 1/2 difference

= (High level – low level)/2

= (0.69-0.79) / 2 = -0.05

EUDRAGIT RS100 x EC = 1/2 difference

= (High level – low level)/2

= (2.085-2.105)/2

= -0.01

EUDRAGIT RS100 x HPMC x EC = 1/2 difference

= (High level – low level) / 2

= (2.045-2.145) / 2

= -0.050

All these interpretations and implications of polymer characteristics over release profile were supported statistically and the results of main effects, interactive (two and three way) effects, were enlisted in Table. 35

Table. 35

EFFECT	ESTIMATE
Average	+23.1
Main effects	
Effect of EC	+2.095
Effect of Eudragit RS100	+1.15
Effect of HPMC K4M	+0.74
Two factor interactions	
Eudragit RS100 X HPMC K4M	-0.05
HPMC K4M X EC	-0.175
Eudragit RS100 X EC	-0.01
Three factor Interactions	
Eudragit RS100 X HPMC K4M X EC	-0.50

The drug release was retarded by increasing the polymer concentration due to increased viscosity and strength of gel matrix formed due to EUDRAGIT RS100 and HPMC K4M and low water permeability of EC. This swelling of EUDRAGIT RS100 is independent on medium pH, which forms hydrogen bonds with imbibing water and also holds water inside the gel matrix. Increasing the amount of HPMC K4M also forms a gel network and there the drug diffusion is controlled by penetration of liquid through the gelled network and there by increasing the $t_{80\%}$ value.

Since the design was optimized statistically using 2^3 factorial designs it is possible to authenticate the design by selecting an extra design check point residing within the influential matrix space and verifying the proximity of predicted response to the observed one. This was done by constructive polynomial equation of linear interactive model type using pertinent statistical calculations listed in following pages.

Table: 36

YATES ALGORITHM

Signs to calculate effects in a 2^3 Factorial Experiment

Factor Combination	Level of Factor in Experiment			Interaction				Total
	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	$X_1X_2X_3$	
F_1	-1	-1	-1	1	1	1	-1	-1
F_2	1	-1	-1	-1	-1	1	1	1
F_3	-1	1	-1	-1	1	-1	1	1
F_4	1	1	-1	1	-1	-1	-1	1
F_5	-1	-1	1	1	-1	-1	1	1
F_6	1	-1	1	-1	1	-1	-1	1
F_7	-1	1	1	-1	-1	1	1	1
F_8	1	1	1	1	1	1	1	1
	+ factor at high level - factor at low level			Multiply signs of factors to obtain signs for interaction terms in				

		combination	

Basic Polynomial equation

$$Y = B_0 + B_1(x_1) + B_2(x_2) + B_3(x_3) + B_{12}(x_1x_2) + B_{13}(x_1x_3) + B_{23}(x_2x_3) + B_{123}(x_1x_2x_3)$$

Calculation of coefficient

$$\mathbf{B_0} = ((1)*(Y1) + (1)*(Y2) + (1)*(Y3) + (1)*(Y4) + (1)*(Y5) + (1)*(Y6) + (1)*(Y7) + (1)*(Y8)) / 8$$

$$= ((1*19.80) + (1*22.05) + (1*21.23) + (1*23.27) + (1*20.86) + (1*22.82) + (1*21.75) + (1*23.88)) / 8$$

$$= 21.9575$$

$$\mathbf{B_1} = ((-1)*(Y1) + (1)*(Y2) + (-1)*(Y3) + (1)*(Y4) + (-1)*(Y5) + (1)*(Y6) + (-1)*(Y7) + (1)*(Y8)) / 8$$

$$= \{(-1)*(19.80) + (1)*(22.05) + (-1)*(21.23) + (1)*(23.27) + (-1)*(20.86) + (1)*(22.82) + (-1)*(21.75) + (1)*(23.88)\} / 8$$

$= 1.0475$

$\mathbf{B_2} = ((-1)*(Y1) + (-1)*(Y2) + (1)*(Y3) + (1)*(Y4) + (-1)*(Y5) +$

$(-1)*(Y6) + (1)*(Y7) + (1)*(Y8)) / 8$

$= ((-1)*(19.80) + (-1)*(22.05) + (1)*(21.23) + (1)*(23.27) + (-1)*(20.86) +$

$(-1)*(22.82) + (1)*(21.75) + (1)*(23.88)) / 8$

$= 0.575$

$\mathbf{B_3} = ((-1)*(Y1) + (-1)*(Y2) + (-1)*(Y3) + (-1)*(Y4) + (1)*(Y5) +$

$(1)*(Y6) + (1)*(Y7) + (1)*(Y8)) / 8$

$= ((-1)*(19.80) + (-1)*(22.05) + (-1)*(21.23) + (-1)*(23.27) + (1)*(20.86) +$

$(1)*(22.82) + (1)*(21.75)+(1)*(23.88)) / 8$

$= 0.37$

$\mathbf{B_{12}} = ((1)*(Y1) + (-1)*(Y2) + (-1)*(Y3) + (1)*(Y4) + (1)*(Y5)+$

(-1)*(Y6) + (-1)*(Y7) + (1)*(Y8)) / 8

= ((1)*(19.80)+(-1)*(22.05)+(-1)*(21.23)+(1)*(23.27)+(1)*(20.86)+

(-1)*(22.82)+(-1)*(21.75) + (1)*(23.88)) /

= -0.005

$\mathbf{B_{13}}$ = ((1)*(Y1)+(-1)*(Y2)+(1)*(Y3)+(-1)*(Y4)+(-1)*(Y5)+

(1)*(Y6) + (-1)*(Y7)+(1)*(Y8)) / 8

= ((1)*(19.8)+(-1)*(22.05)+(1)*(21.23)+(-1)*(23.27)+

(-1)*(20.86)+(1)*(22.82)+ (-1)*(21.75) + (1)*(23.88)) / 8

= -0.025

$\mathbf{B_{23}}$ = ((1)*(Y1)+(1)*(Y2)+(-1)*(Y3)+(-1)*(Y4)+(-1)*(Y5)+

(-1)*(Y6) + (1)*(Y7) + (1)*(Y8)) / 8

= ((1)*(19.8) + (1)*(22.05) + (-1)*(21.23) + (-1)*(23.27) + (-1)*(20.86)+

(-1)*(22.82) + (1)*(21.75)+(1)*(23.88)) / 8

= -0.0875

$\mathbf{B_{123}}$ = ((-1)*(Y1) + (1)*(Y2) + (1)*(Y3) + (-1)*(Y4) + (1)*(Y5) +

(1)*(Y6) + (1)*(Y7) + (1)*(Y8)) / 8

= ((-1)*(19.8) + (1)*(22.05) + (1)*(21.23) + (-1)*(23.27) + (1)*(20.86) +

(1)*(22.82) + (1)*(21.75) + (1)*(23.88)) / 8

= 5.485

Apply the above values in the basic polynomial equation.

Let us consider transformed values

$x_1 = 0.5$

$x_2 = 0.5$

$x_3 = 0.5$

Actual polynomial Equation

Y = (21.9575) + (1.0475)(0.5) + (0.575) (0.5) + (0.37)(0.5) + (-0.005)(0.5*0.5) +

(-0.025)(0.5*0.5) + (-0.0875) (0.5*0.5) + (5.485) (0.5*0.5*0.5)

= 23.416875

CALCULATION FOR CHANGING THE TRANSFORMED PROPORTIONS TO THE ACTUAL POLYMER COMPOSITION

Table. 37

Signs to calculate effects in a 2^3 Factorial Experiment

Factor	Low level	High level	Average	1/2 of difference of two values
EC	750	1000	875	125
EUDRAGIT RS100	100	200	150	50
HPMC K4M	200	300	250	50

Average = (low level+ high level) / 2

½ of the difference of two values = (High level- low level) / 2

$$\text{Transformed value} = \frac{x - \text{Average of two values}}{\text{One half of difference of two values}}$$

x_1 =Conc. of EC

x_2 = Conc. of EUDRAGIT RS100

x_3= Conc. of HPMC K4M

For EC

$$\frac{x_1 - 875}{125} = 0.5$$

$$x_1 = 937.5$$

For EUDRAGIT RS 100

$$\frac{x_2 - 150}{50} = 0.5$$

$$x_2 = 175$$

For HPMC K4M

$$\frac{x_3 - 250}{50} = 0.5$$

$$x_3 = 275$$

Variables in extra design check point

x_1 = 937.5mg of EC

x_2 = 175mg of EUDRAGIT RS100

x_3 = 275mg of HPMC K4M

Table. 38

IN VITRO DRUG RELEASE DATA FOR EXTRA DESIGN CHECK POINT OF *F9- 1

Time in hrs	Absorbance	Concentration in µg/ml	Amount released in mg/ml	Cumulative amount released in 900ml(mg)	Cumulative % drug release
0	0.000	0.0000	0.0000	0.0000	0.00
1	0.033	1.5739	0.0079	7.0827	9.44
2	0.071	3.3863	0.0169	15.2542	20.34
3	0.032	1.4947	0.0075 21.9806	29.31	
4	0.054	2.5224	0.0126	26.6199	35.49
5	0.071	3.3165	0.0166	30.2185	40.29
6	0.082	3.8303	0.0192	32.5638	43.42
7	0.096	4.4842	0.0224	35.5449	47.39
8	0.115	5.3717	0.0269	39.5835	52.78
9	0.122	5.6987	0.0285	41.1086	54.81
10	0.133	6.2125	0.0311	43.4778	57.97
11	0.141	6.5862	0.0329	45.2215	60.30
12	0.147	6.8665	0.0343	46.5486	62.06
13	0.152	7.1000	0.0355	47.6682	63.56
14	0.155	7.2402	0.0362	48.3698	64.49
15	0.161	7.5204	0.0376	49.7034	66.27
16	0.163	7.6139	0.0381	50.1990	66.93
17	0.169	7.8941	0.0395	51.5363	68.72
18	0.174	8.1277	0.0406	52.6663	70.22
19	0.180	8.4079	0.0420	54.0087	72.01
20	0.188	8.7816	0.0439	55.7744	74.37
21	0.195	9.1086	0.0455	57.3336	76.44
22	0.200	9.3422	0.0467	58.4757	77.97
23	0.203	9.4823	0.0474	59.1997	79.93
24	0.212	9.9027	0.0495	61.1863	83.04

Figure. 44

Invitro release of Venlafaxine HCl from Formulation F9-1

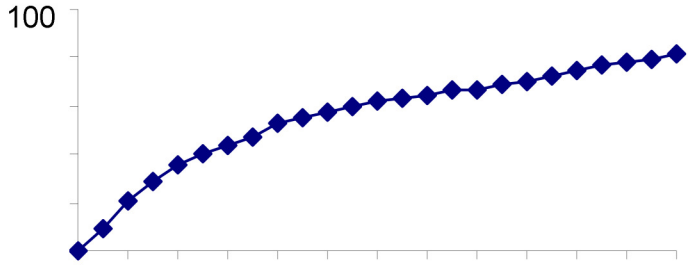


Figure. 45

Higuchi plot of formulation F9-1

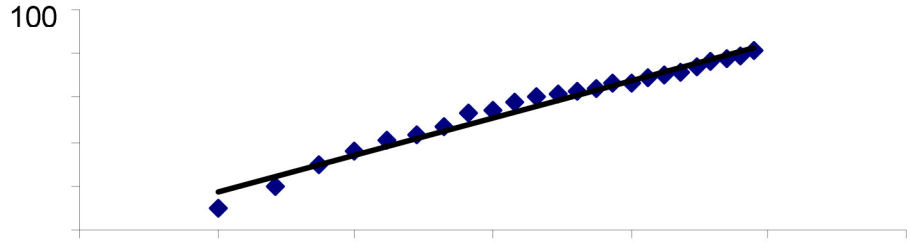


Figure. 46

Korzemayer peppas plot for formulation F9-1

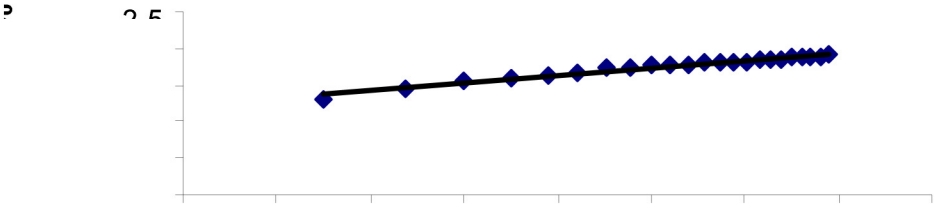


Figure. 47

First order plot for formulation F9-1

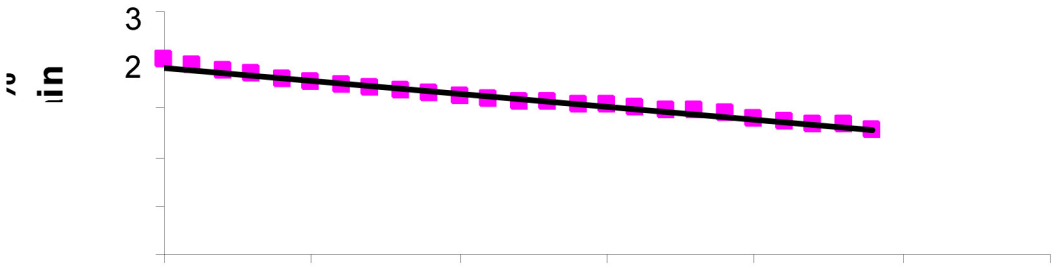


Table. 39

IN VITRO DRUG RELEASE DATA FOR EXTRA DESIGN CHECK POINT OF *F9- 2

Time in hrs	Absorbance	Con. in mg/ml	Amount released in mg	Cumulative amount released in mg	Cumulative % drug release
0	0.000	0.0000	0.0000	0.0000	0.00
1	0.033	1.5739	0.0079	7.0827	9.44
2	0.072	3.4340	0.0172	15.4688	20.63
3	0.034	1.5882	0.0079	22.6156	30.15
4	0.053	2.4757	0.0124	26.6252	35.50
5	0.071	3.3165	0.0166	30.4336	40.58
6	0.080	3.7369	0.0187	32.3585	43.14
7	0.095	4.4375	0.0222	35.5489	47.40
8	0.112	5.2316	0.0262	39.1666	52.22
9	0.120	5.6053	0.0280	40.9005	54.53
10	0.130	6.0724	0.0304	43.0586	57.41
11	0.140	6.5395	0.0327	45.2213	60.30
12	0.143	6.6796	0.0334	45.9173	61.22
13	0.152	7.1000	0.0355	47.8758	63.83
14	0.158	7.3803	0.0369	49.2080	65.61
15	0.161	7.5204	0.0376	49.9124	66.55
16	0.163	7.6139	0.0381	50.4080	67.21
17	0.167	7.8007	0.0390	51.3250	68.43
18	0.171	7.9875	0.0399	52.2438	69.66

19	**0.180**	**8.4079**	**0.0420**	**54.2154**	**72.29**
20	**0.184**	**8.5948**	**0.0430**	**55.1403**	**73.52**
21	**0.192**	**8.9685**	**0.0448**	**56.9078**	**75.88**
22	**0.200**	**9.3422**	**0.0467**	**58.6791**	**78.24**
23	**0.201**	**9.3889**	**0.0469**	**58.9827**	**79.64**
24	**0.213**	**9.9494**	**0.0497**	**61.5990**	**83.55**

Figure. 48

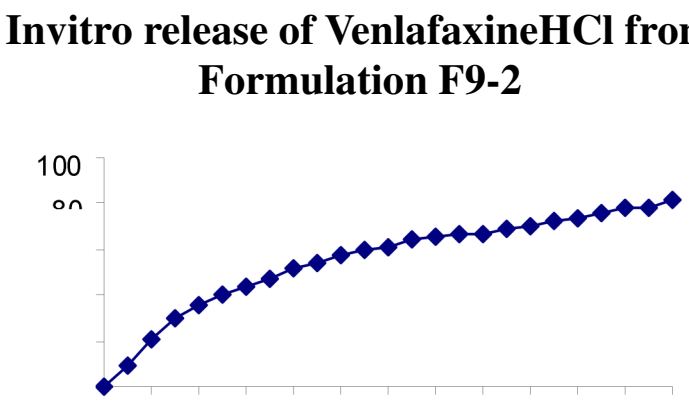


Figure. 49

Higuchi plot of formulation F9-2

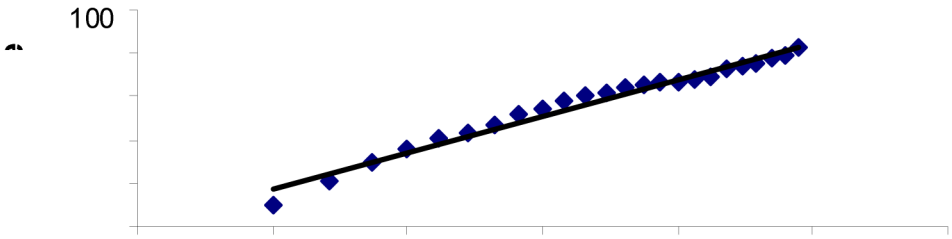


Figure. 50

Korzemayer peppas plot for formulation F9-2

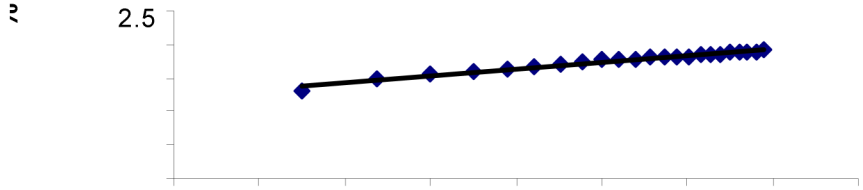


Figure. 51

First order plot for formulation F9-2

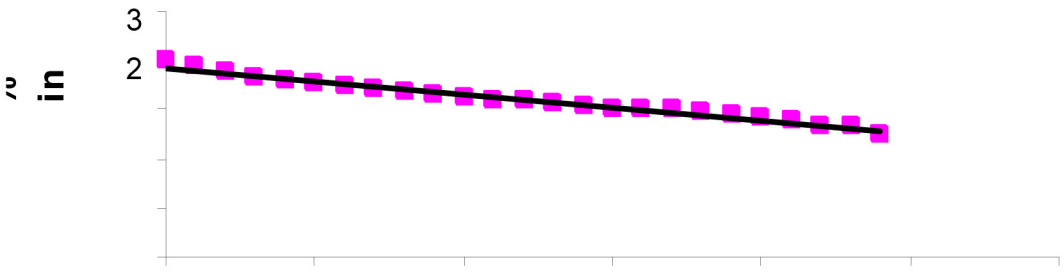


Table 40

IN VITRO DRUG RELEASE DATA FOR EXTRA DESIGN CHECK POINT OF *F9- 3

Time in hrs	Absorbance	Con. in mg/ml	Amount released in mg	Cumulative amount released in mg	Cumulative % drug release
0	0.000	0.0000	0.0000	0.0000	0.00
1	0.035	1.6693	0.0083	7.5119	10.02
2	0.072	3.4340	0.0172	15.4698	20.63
3	0.035	1.6349	0.0082	22.8267	30.44
4	0.054	2.5224	0.0126	26.8369	35.78
5	0.073	3.4099	0.0170	30.8559	41.14
6	0.085	3.9704	0.0199	33.4123	44.55
7	0.096	4.4842	0.0224	35.7642	47.69
8	0.117	5.4652	0.0273	40.2232	53.63
9	0.125	5.8388	0.0292	41.9595	55.95
10	0.135	6.3060	0.0315	44.1199	58.83
11	0.142	6.6329	0.0332	45.6543	60.87
12	0.147	6.8665	0.0343	46.7716	62.36
13	0.157	7.3336	0.0367	48.9423	65.26
14	0.161	7.5204	0.0376	49.8564	66.48
15	0.165	7.7073	0.0385	50.7724	67.70
16	0.168	7.8474	0.0392	51.4801	68.64
17	0.171	7.9875	0.0399	52.1891	69.59
18	0.174	8.1277	0.0406	52.8996	70.53

19	**0.181**	**8.4547**	**0.0423**	**54.4523**	**72.60**
20	**0.189**	**8.8283**	**0.0441**	**56.2184**	**74.96**
21	**0.195**	**9.1086**	**0.0455**	**57.5679**	**76.76**
22	**0.199**	**9.2954**	**0.0465**	**58.4998**	**78.00**
23	**0.203**	**9.4823**	**0.0474**	**59.4335**	**79.24**
24	**0.214**	**9.9961**	**0.0500**	**61.8405**	**82.76**

Figure 52

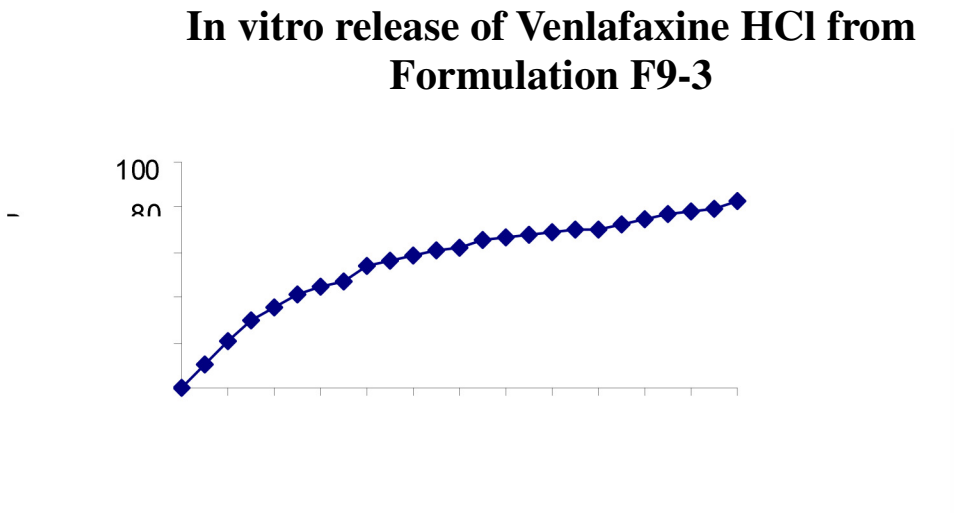


Figure. 53

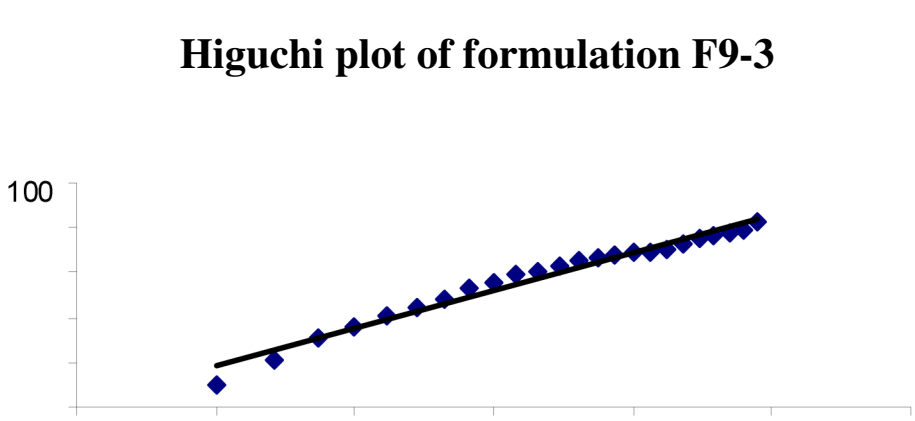


Figure. 54

Korzemayer peppas plot for formulation F9-3

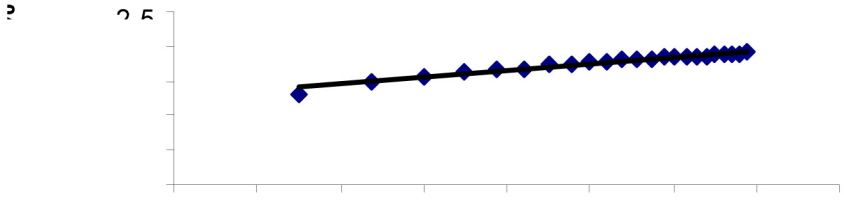


Figure. 55

First order plot for formulation F9-3

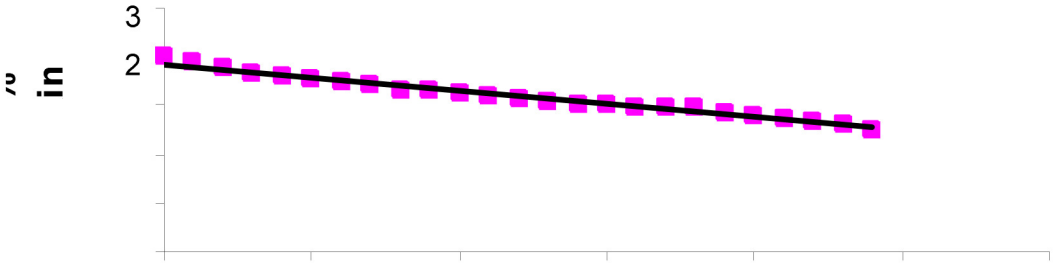


Table. 41

INVITRO DRUG RELEASE PROFILE OF EXTRA DESIGN CHECK POINT

Time in hrs	Cumulative percentage drug release			Meam cumulative % drug release ±SD
	Trial 1	Trial 2	Trial 3	
0	0.00	0	0.00	0±0
1	9.16	9.73	10.30	9.73±0.57
2	14.04	14.62	15.19	14.61±0.575
3	25.53	26.67	27.80	26.66±1.135
4	29.76	31.71	32.03	31.16±1.228
5	32.32	33.46	34.59	33.45±1.135
6	34.32	35.46	36.60	35.46±1.14
7	38.57	39.71	40.85	39.71±1.14
8	43.11	44.25	45.39	44.25±1.14
9	46.82	47.96	49.10	47.96±1.14
10	49.69	50.84	51.98	50.83±1.145
11	52.01	53.16	54.30	53.15±1.145
12	54.06	55.2	56.35	55.20±1.145
13	55.83	56.97	58.12	56.97±1.145
14	60.40	61.55	62.70	61.55±1.15
15	62.19	63.34	64.48	63.33±1.145

16	**63.69**	**64.84**	**65.99**	**64.84±1.15**
17	**64.92**	**66.07**	**67.23**	**66.07±1.155**
18	**66.71**	**67.87**	**69.02**	**67.86±1.155**
19	**68.51**	**69.67**	**70.82**	**69.66±1.155**
20	**70.87**	**72.03**	**73.18**	**72.02±1.155**
21	**72.68**	**73.83**	**74.99**	**73.83±1.155**
22	**75.05**	**76.2**	**77.36**	**76.20±1.155**
23	**77.42**	**78.58**	**79.74**	**78.58±1.16**
24	**79.24**	**80.4**	**81.56**	**80.4±1.16**

Table. 42

IN VITRO RELEASE KINETIC DATA FOR EXTRA DESIGN CHECK POINT

Formula code	**$t_{80\%}$**	**Zero order**		**First order**		**Higuchi's**	**Korsmeyer-Peppas**	
		K_0	**r**	**K_1**	**r**	**r**	**n**	**r**
F_{9-1}	23.12	2.7948	0.94017	-0.02741	-0.9897	0.9833	0.5833	0.9741
F_{9-2}	22.98	2.8082	0.9393	-0.0271	-0.9900	0.9888	0.5839	0.9736
F_{9-3}	23.20	2.8120	0.9353	-0.274	-0.9888	0.9910	0.5756	0.9746

Figure. 56

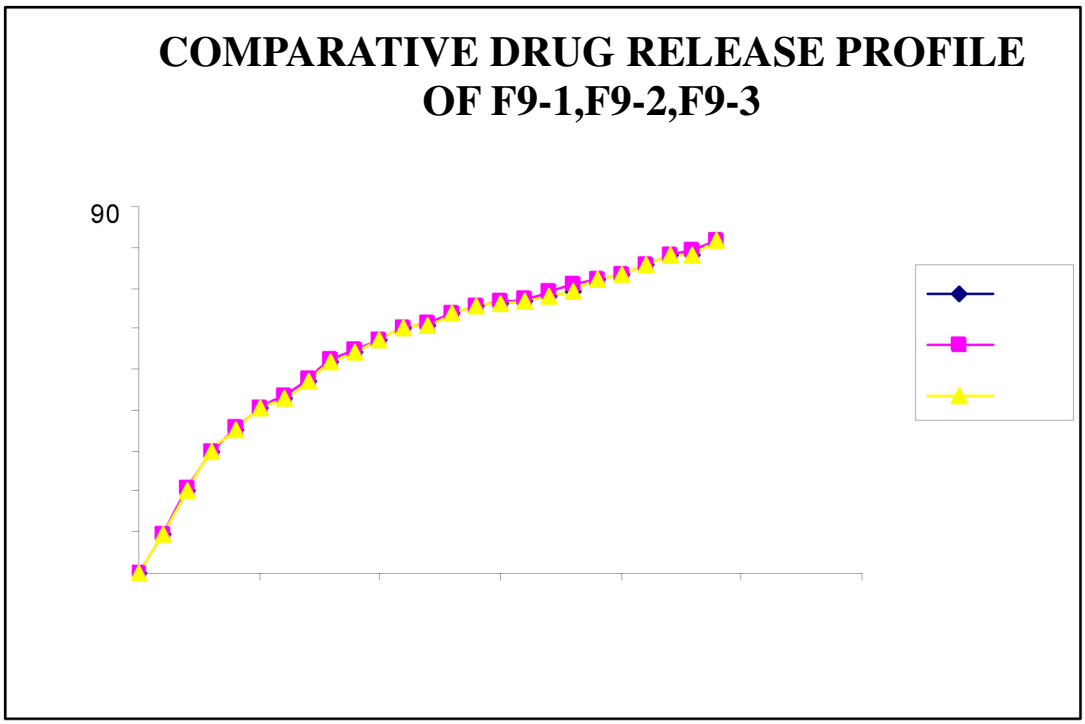


Table. 43

STUDENT T TEST ON EXTRA DESIGN CHECK POINT

Trial code	Predicted T_{80}	Estimated T_{80}
1	23.42	23.12
2	23.42	22.98
3	23.42	23.20

P Value = 0.019037 (<0.05)

Having designed using appropriate statistical calculations, the polynomial equation was used to predict the response that would fulfill the aim of the present study.

By calculating actual polymer concentration from transformed proportions of each variable, the extra design checkpoint formulation was designed. Predicted to exhibit $t_{80\%}$ of 23.42 hours, the extra design checkpoint batch was observed to have $t_{80\%}$ of 23.12, 22.98, 23.20 hours. The statistical insignificance of the difference between the predicted and observed responses (P<0.05 OF student T test shows 95% confidence) not only validate the design adopted for optimization but also confirms usefulness of the polynomial equation in predicting the *in vitro* kinetic parameters.

Table. 44

f_2SIMILARITYFACTORSOFFORMULATIONSOFMUCOADHESIVE MICROSPHERES OF VENLAFAXINE HCL

FORMULATION CODE	f_2 SIMILARITY FACTOR
F1	64.42
F2	80.78
F3	76.33
F4	77.38
F5	88.75
F6	66.86
F7	86.80
F8	66.11

The formulation F_5 (, EC-750 mg Eudragit RS100-100 mg, HPMC K4M-300 mg) was selected as optimized formulation with 82.58% of drug release at 24th hours.

9.0 DISCUSSION

Venlafaxine HCL is serotonine-epinephrine reuptake inhibitor; a potent drug for depressive disorders and anxiety. The oral route is preferred for Venlafaxine HCl but it has only 12.6% oral bioavailability due to high first pass metabolism, and the conventional dosage forms are not recommended because of shorter half life of 5 Hrs

In this present work efforts have been made to develop mucoadhesive microspheres of Venlafaxine HCl using emulsion solvent evaporation technique using Ethyl Cellulose, Eudragit RS100 along with mucoadhesive polymer Hydroxy Propyl Methyl Cellulose K4M. The polymer concentration is the major factor controlling drug release and mucoadhesion

The FTIR spectral analysis showed that there was no appearance or disappearance of any characteristic peaks of pure drug Venlafaxine HCl in the physical mixture of drug and polymer, which confirms the absence of chemical interaction between drug and polymers. The DSC spectral analysis also reveals the same.

The percentage yield of microspheres of all formulations was in the range of 43.68% to 91.82%. The microsphere prepared by this method was found to be discreet, spherical, free flowing and it was observed by Scanning Electron Microscopy (SEM) Figures.41,42,43. The microspheres were uniform in size with a size range of 65.60µm to 105.30µm. The angle of repose revealed that the microspheres of all the batches had good flow characteristics and flow rates. Table 20. The bulk density was in the range of 0.4880 to 0.5913 were shown in Table 21

The drug content determination showed that even if the polymer composition was changed the process was highly efficient to give microspheres having maximum drug loading. Table 22. The entrapment efficiency was in the range of 68.38% to 93.08%. Table No 23.Microspheres of Venlafaxine HCl exhibited good mucoadhesive properties in the in vitro wash of test .The test result of wash off test were shown in the Table 24,25 The F5 formulation has more adhesive strength than others. The prepared tablets were then subjected to dissolution test for evaluating the in vitro drug release. The dissolution studies were carried out in 900 ml phosphate buffer pH 6.8 in USP basket type dissolution apparatus at 50 rpm and 37 ± 0.5^{O} C. The results of the dissolution studies indicate that the polymer concentration is having a substantial effect on the drug release from the microspheres. The invitro drug release was found to be 80.4 to 87.89(Table 26 to 33)

The statistical analysis of the factorial design batches was performed by multiple linear regression analysis using Microsoft Excel. The results depicted in Table 36 clearly indicate that the response Y is strongly dependent on the selected independent variables, as shown by the wide variation among the 8 batches (F1-F8). The polynomial equation was used to predict the response that would fulfill the aim of the present study. By calculating actual polymer concentration from transformed proportions of each variable, the extra design checkpoint formulation was designed. Predicted to exhibit $t_{80\%}$ value of 23.42, the extra design checkpoint batch was observed to have $t_{80\%}$ value of the 23.12, 22.98 and 23.20 hrs in the three trials respectively (Table. 43).

The observed $t_{80\%}$ was then compared with the predicted response by using student's t test. The p value of the students t test was found to be 0.0190, this statistical insignificance of the difference between the predicted and observed responses not only

validate the design adopted for optimization but also confirms usefulness of the polynomial equation in predicting the *in vitro* kinetic parameters.

Along with the closeness of the $t_{80\%}$ of the formulation batches to that of the extra design check point batch, similarity factor *f2* was also used for the purpose of selecting the optimized formulation. Out of all of the batches, formulation F5 with low levels of X1,X2,and high level of X3 was having maximum *f2* value of 88.75. Also formulation F5was having $t_{80\%}$ of 20.86, which is the closest among all the formulations to the $t_{80\%}$ of the extra design checkpoint batch. Based on these results we can say that Formulation F5 was found to be the optimized formulation.

In order to understand the complex mechanism of drug release from the mucoadhesive microspheres, the *in vitro* **Venlafaxine Hcl.** release data were fitted to korsmeyer-peppa's release model and interpretation of release exponent values (n) enlightens in understanding the release mechanism from the dosage form. The release exponent values thus obtained were ranged from 0.5763 to 0.6692are shown in Table.34. All the formulations exhibited anomalous (non-fickian transport) diffusion mechanism. The drug release was diffusion controlled as the plot of Higuchi's model was found to be linear ($r > 0.983$). These formulations are also showed as highest 'r ' values of first order kinetics indicating the **Venlafaxine HCl.** release from these mucoadhesive microspheres were by diffusion.

9.0 CONCLUSION

Mucoadhesive microspheres had shown excellent bioavailability and loading efficiency with Venlafaxine HCl. The polymer concentration is a major factor affecting the release and mucoadhesion strength of the prepared microspheres. The observed response ($t_{80\%}$) is close agreement with the predicted $t_{80\%}$ value there by demonstrating the feasibility of the optimization procedure in developing mucoadhesive microspheres containing All the formulations exhibited anomalous (non-fickian transport) diffusion mechanism and follow first order kinetic. The formulation with EC-750 mg, Eudragit RS100 100 mg and HPMC K4M-300 mg was selected as optimized formulation; with 82.58% of drug release at 24^{th} hours.

Finally it is concluded that with limited number of experiments an optimized formulation with target release and good mucoadhesion can be developed with appropriate statistical experimental design.

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